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THE EFFECT OF DIETARY PROTEIN CONTENT UPON THE NITROGEN RETENTION AND WEIGHT GAIN PRODUCED BY THE HYPOPHYSEAL GROWTH HORMONE

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INTRODUCTION

THE FACT that hypophyseal extracts rich in growth hormone produce nitrogen retention has been shown repeatedly by many workers (Teel and Watkins, 1929; Teel and Cushing, 1930; Gaebler, 1933; Lee and Shaffer, 1934; Shaffer and Lee, 1935; Marx *et al.*, 1942; and Young, 1945). Purified growth hormone has the same effect in alloxan-diabetic rats (Bennett and Li, 1947), and in rats with increased nitrogen excretion following bilateral femur fracture (Bennett, Applegarth, and Li, 1946). Since the nitrogen retained must be derived from the dietary intake and be incorporated into normal body tissues, it would be reasonable to expect that the nitrogen retaining effect of a given amount of growth hormone would be influenced by the biological quality and the quantity of protein ingested. In these experiments we have used a standard dose of the growth hormone and have compared the amounts of weight gained and of nitrogen retained by rats fed constant amounts of iso-caloric diets with varying protein content.

METHODS

"Plateaued" female rats of the Long-Evans strain aged seven to ten months were used. The animals were fed purified diets in which alcohol-

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washed casein was the protein source and which contained optimal amounts of all known dietary factors. All diets contained 8 per cent fat, 4 per cent salts (Hegsted *et al.*, 1941), and 0.5 per cent liver fraction powder. The casein content of the five diets was 6 per cent, 12 per cent, 18 per cent, 24 per cent, and 48 per cent respectively, and the remainder of each diet consisted of sucrose, so that all diets were iso-caloric.

To each Kg. of diet were added thiamine chloride 5 mg., pyridoxine hydrochloride 5 mg., riboflavin 10 mg., p-aminobenzoic acid 10 mg., nicotinic acid 20 mg., calcium pantothenate 50 mg., inositol 500 mg., choline chloride 1 gm., and 2 methyl 1,4 naphthoquinone 5 mg. Each week each animal was fed the following fat soluble vitamins in 650 mg. of corn oil (Mazola): 6 mg. of alpha-tocopherol, 115 chick units of Vitamin D, 800 U.S.P. units of Vitamin A, one-seventh of the weekly amount being added to the daily feeding. The 24 per cent casein diet without liver fraction powder has been shown to support growth equal or superior to that produced on optimal stock diets of natural foods (Nelson and Evans, 1947). The "liver fraction powder" (Lederle 7-5293) has subsequently been added to supply "unknown Vitamin B_2 factors" and to insure completeness of the diet according to current concepts of nutrition.

It was found that 12 gm. of diet, weighed to 0.1 gm., was the maximum amount that each animal could be relied upon to eat per day, and this amount of diet was fed daily to each animal in each experiment. It should be emphasized that in all experiments all animals received the same caloric intake, no attempt being made to compensate for the varying specific dynamic actions of the diets. No rat was used in more than one experiment. The animals were weighed at the same time daily, immediately before being fed.

After an adaptation period of from three to seven weeks on the purified diets, the rats were placed in individual screen-bottomed cages. Urines were collected through acid-moistened, ribbed glass funnels into wide-mouth Erlenmeyer flasks containing approximately 10 cc. of 1N H_2SO_4, feces and hair being separated from the urine upon perforated porcelain discs. Funnels were washed with distilled water and urines collected at 48-hour intervals except during injection periods when urines were collected at 24-hour intervals. Urines were analyzed for nitrogen content by the micro-Kjeldahl method and are reported as 24-hour nitrogen excretion.

At each dietary level the weights and urinary nitrogen excretions of each of ten rats were determined for ten days prior to injection. Injection periods of five days were used during which half of the animals received 0.5 mg.of growth hormone intraperitoneally twice daily while the others were similarly injected with an equal amount of serum albumen. The hormone preparation used was prepared according to the previously published method (Li, Evans, and Simpson, 1945). Following the period of injections, the animals were similarly studied for a second control period of ten days duration.

EXPERIMENTAL DATA

The effect of the hypophyseal growth hormone upon the body weight and urinary nitrogen excretion of rats fed the 6 per cent casein diet is reported elsewhere (Gordan, Bennett, Li and Evans, 1947). It was shown that on the 6 per cent casein diet growth hormone produced

nitrogen retention without significant gain in weight. When methionine was added to the diet, growth hormone produced nitrogen retention and concomitant weight gain. With the methionine supplement five control animals showed an average weight increase of 4 gm. (range 3 to 7) in five days while five treated animals showed an average

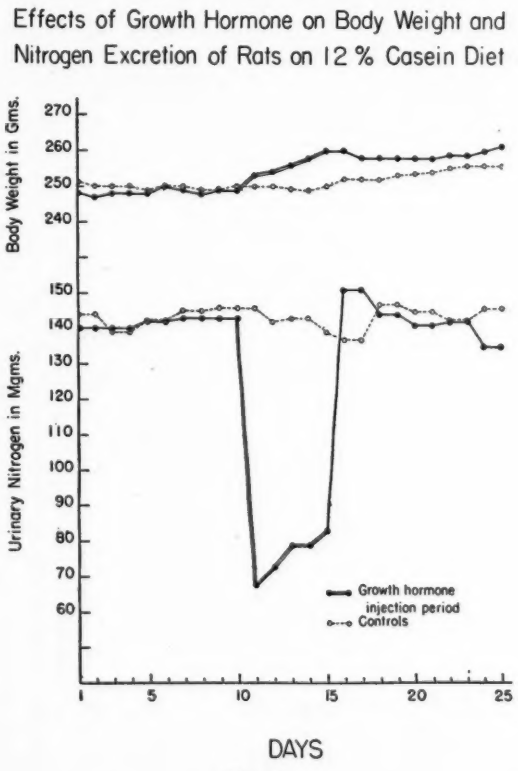

FIG. 1

weight gain of 13 mg. (range 10 to 15). The difference in the increment of weight between the control and growth hormone injected rats was calculated as a net gain of 9 gm. ascribed to the action of growth hormone. The control rats excreted an average of 87 mg. of nitrogen per day of injection while the growth hormone treated rats excreted only 48 mg. per animal per day. The difference was calculated as a net retention of 39 mg. of nitrogen per animal per day of injection.

With dietary casein contents of 12 per cent, 18 per cent, 24 per cent, and 48 per cent, no amino acid supplements were used. The data and their statistical analyses are presented in Table 1. A graphic picture of the day by day body weight changes and nitrogen excretion

TABLE 1. EFFECT OF GROWTH HORMONE ON URINARY NITROGEN EXCRETION AND BODY WEIGHT OF RATS

Diet	Group	Daily nitrogen excretion				Nitrogen retention produced by growth hormone†	Change in weight during treatment period	Increment in body weight due to growth hormone‡	Theoretical increase in body weight§
		Ten day preinjection period	Five day injection period		Ten day post-injection period				
		mgs.	mgs.	p*	mgs.	mgs.	gms.	gms.	
6% casein plus methionine	Controls	77 ± 2.3	87 ± 2.4		92 ± 2.0		+ 4		
	Treated	68 ± 2.3	48 ± 2.3	<0.01	93 ± 3.9	+39	+13	+ 9	6.1
12% casein	Controls	143 ± 2.9	143 ± 2.7		146 ± 2.5		0		
	Treated	142 ± 2.2	76 ± 2.0	<0.01	143 ± 2.3	+67	+11	+11	10.5
18% casein	Controls	205 ± 1.7	206 ± 2.3		206 ± 1.6		+ 3		
	Treated	206 ± 1.6	114 ± 2.0	<0.01	209 ± 1.6	+92	+18	+15	14.4
24% casein	Controls	369 ± 7.5	371 ± 9.6		350 ±14.5		0		
	Treated	338 ± 7.7	252 ±11.1	<0.01	372 ± 8.9	+119	+21	+21	18.6
48% casein	Controls	716 ±22.2	687 ±19.8		647 ±27.0		+ 2		
	Treated	723 ±14.3	566 ±15.6	<0.01	740 ±23.6	+121	+18	+18	18.8

* From Fisher's (1946) table of t comparing the significance of the mean excretion of the treated and control animals during the injection period.

† The difference between the excretion of the control and treated animals during the treatment period.

‡ The difference between the weight change of the treated and control animals.

§ Calculated as explained in the text.

is presented in Figures 1 to 4. By reference to Table 1 it will be noted that the average daily retention of nitrogen produced by the growth hormone was 67 mg., 92 mg., 119 mg., and 121 mg. for the groups fed 12 per cent, 18 per cent, 24 per cent, and 48 per cent casein diets re-

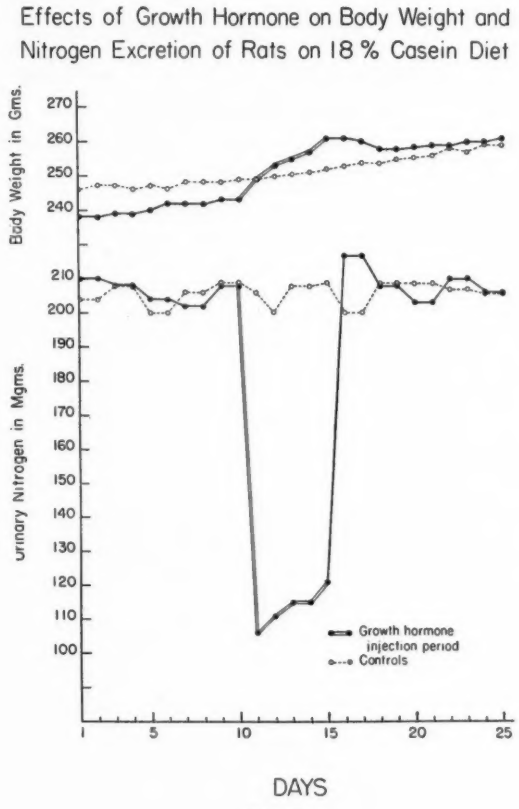

FIG. 2

spectively. The amount of weight gain likewise increased with the increasing dietary protein content until the 24 per cent casein level was reached.

DISCUSSION

It would appear from our data that there is a correlation between the amount of nitrogen retained and the amount of body weight gained during the five-day injection period. F. G. Young (1945) has shown by careful carcass analysis that the nitrogen retained, calculated as protein plus protein-bound water, accounts quantitatively for the amount of weight gained. Assuming that the tissue formed is 20 per cent protein and that the protein is 16 per cent nitrogen, a theo-

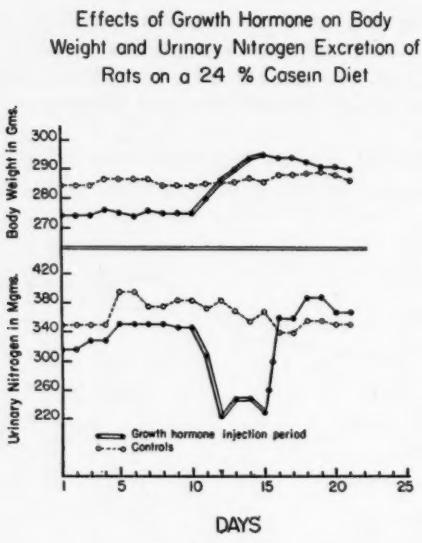
Effects of Growth Hormone on Body Weight and Urinary Nitrogen Excretion of Rats on a 24 % Casein Diet
Body Weight in Gms.
300
290
280
270
Urinary Nitrogen in Mgms.
420
380
340
300
260
220
Growth hormone injection period
Controls
1
5
10
15
20
25
DAYS

FIG. 3

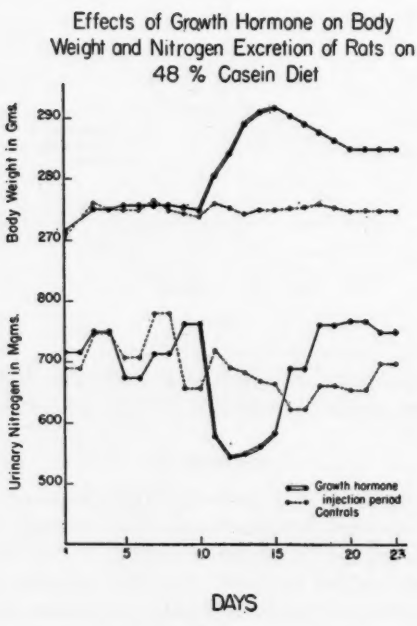
Effects of Growth Hormone on Body Weight and Nitrogen Excretion of Rats on 48 % Casein Diet
Body Weight in Gms.
290
280
270
Urinary Nitrogen in Mgms.
800
700
600
500
Growth hormone injection period
Controls
5
10
15
20
DAYS

FIG. 4

retical weight increase can be calculated which corresponds strikingly with the amount of weight gain actually observed in the experiments (Table 1).

The relation of the body weight gains and the degree of nitrogen retention to the dietary protein content are graphed in Figure 5. It will be observed that the amount of nitrogen retention and increment in body weight produced by a standard dose of the growth hormone under these experimental conditions both increase in a linear fashion

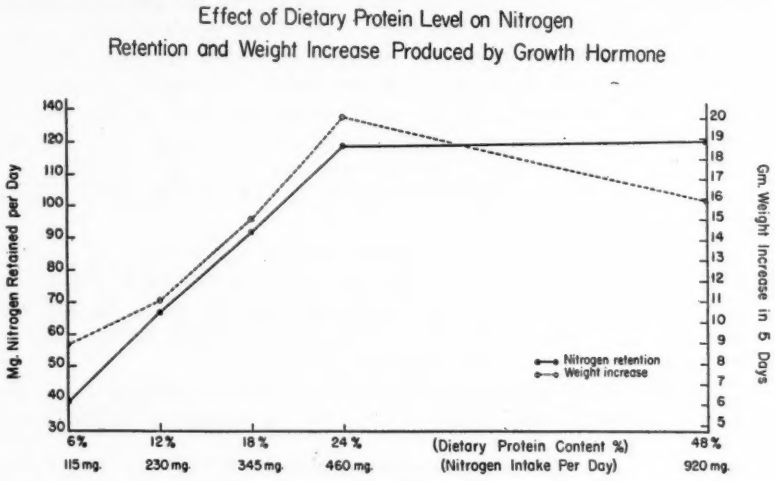

FIG. 5

from the 6 per cent to the 24 per cent casein diet and then fail to show any further increase when the dietary protein content is doubled.

These experiments have shown a characteristic pattern of response to the growth hormone which perhaps would be present only under these experimental conditions. Following the injection of growth hormone there is a prompt decrease in the urinary nitrogen excretion, usually maximal in the first 24 hours. Decreased urinary nitrogen excretion persists during the entire five-day injection period. Following cessation of injection the urinary nitrogen excretion promptly returns to the pre-injection level or is slightly higher.

Concomitant with the nitrogen retention is a proportionate increase in body weight. While the animals usually show the most rapid weight gain in the first 24 hours, they continue to gain weight throughout the injection period. Following cessation of injection the treated rats usually lose approximately 20 per cent to 30 per cent of the gained weight by the fourth post-injection day, then regain this in part or wholly to plateau once more on the tenth post-injection day at a level at or slightly below the maximal weight attained. The weight loss may be at least to some extent attributable to the excretion of some of the

previously retained nitrogen. Following cessation of injection the urinary nitrogen excretion returns to pre-injection levels or is slightly higher but never markedly so.

SUMMARY

The action of a standard daily dose of growth hormone for a five-day injection period on the body weight and urinary nitrogen excretion of rats fed complete diets is characteristically as follows: (a) the nitrogen excretion promptly decreases, usually to the greatest extent in the first 24 hours; (b) the body weight promptly increases; (c) upon cessation of injection, the nitrogen excretion promptly returns to the pre-injection level or is slightly higher; (d) upon cessation of injection approximately 20 per cent to 30 per cent of the weight gained is lost, the weight usually reaching the lowest level on the fourth post-injection day. The rats then regain weight to "plateau" again on the tenth post-injection day at or near the maximal weight attained.

The amount of nitrogen retained and the increment of weight gained under the influence of a five-day injection period of the hypophyseal growth hormone both vary concordantly with the dietary protein content. The 24 per cent casein diet is optimal for the dose of growth hormone which was employed.

The amount of nitrogen retained, calculated as protein plus protein-bound water, corresponds closely to the observed weight gain.

ACKNOWLEDGMENT

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CHEMICAL CHANGES IN THE BLOOD COMPOSITION OF CHICKENS AND TURKEYS FED SYNTHETIC ESTROGENS

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PHYSIOLOGICAL changes in the lipid metabolism of the domestic fowl produced by the administration of estrogens have been referred to frequently in the literature (Entenman, Lorenz and Chaikoff 1938; 1940; Lorenz, Chaikoff and Entenman 1938). Lorenz and his coworkers, however, were the first to suggest a practical application of these facts in fattening market poultry through the use of estrogen pellet implants (Lorenz, 1943; 1944). Articles by Jaap and Thayer (1945) further increased the interest among poultrymen by calling attention to the possibility of fattening poultry by administering certain synthetic estrogens orally rather than by pellet implants as had been suggested by Lorenz. These articles also called attention to the fact that the physiological action of various synthetic estrogens was not necessarily similar for all poultry, since the order of estrogen activity was not parallel for chickens and turkeys.

In conducting fattening tests, a guide was needed to determine the amount of estrogen to be administered and the length of the fattening period. This need was best met by chemical analyses of blood and tissue of the experimental birds. During the past four years thousands of chemical determinations have been completed under the supervision of the authors of the present paper. Articles have been published summarizing some of the outstanding observations, but it is felt that more complete data should be on record. This paper reports the average blood analyses for fryers, broilers, cockerels, mature hens, cocks, and turkeys which had been fed fattening rations containing different levels of synthetic estrogens.

EXPERIMENTAL

Some work was done with market poultry in which solutions of estrogens were injected subcutaneously and estrogen pellet implants were made. Most of the results herein reported are from chickens and turkeys which received the estrogens dissolved in an oil vehicle and administered orally as an ingredient of a balanced basal fattening ration. Feeding was conducted under practical conditions, but with

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the fowls confined in poultry houses where feed consumption data could be obtained. In some cases individual cages or fattening batteries were used. The chickens and turkeys were of various breeds selected from the Oklahoma Agricultural Experiment Station flocks.

Blood for analysis was drawn either by heart puncture or from wing veins in a hypodermic syringe and delivered into containers which had been previously coated by evaporating a solution of lithium citrate. In some cases samples were centrifuged to measure

TABLE 1. CHICKEN BLOOD ANALYSES
Average of 20 Pooled Samples from Mature Hens

Cells in whole blood, per cent	27.4
Plasma in whole blood, per cent	72.6
Glucose, mg./100 ml. blood	214.7
Non-protein nitrogen, mg./100 ml. blood	33.3
Urea, mg./100 ml. blood	5.7
Uric acid, mg./100 ml. blood	4.48
Creatinine, mg./100 ml. blood	2.13
Total phosphorus of whole blood, mg./100 ml.	122.3
Total phosphorus of cells, mg./100 ml.	284.2
Total phosphorus of plasma, mg./100 ml.	35.7
Inorganic phosphorus of plasma, mg./100 ml.	4.28
Calcium of plasma, mg./100 ml.	20.9
Free cholesterol of whole blood, mg./100 ml.	60.9
Total fatty acid of whole blood, mg./100 ml.	827.5
Carotene of plasma, micrograms/100 ml.	287.0
Vitamin A of plasma, micrograms/100 ml.	71.6

the cell-plasma ratio and to obtain a clear plasma for analysis. It was frequently observed that the blood from birds receiving certain synthetic estrogens had a light yellow color and a layer of fat appeared on top of the hematocrit tube. The plasma and blood were analyzed by the usual clinical methods (Schoenheimer and Sperry, 1934; Stoddard and Drury, 1929; Youngburg and Youngburg, 1930) at daily and weekly intervals during the fattening period and in some cases during a period of time after the estrogen feeding had been discontinued. Space permits reporting only the average results at certain intervals throughout the period, to show general trends.

Chickens

As a basis for later comparison, the average constituents of the blood from chickens consuming the basal ration under normal station conditions was first determined and is recorded in Table 1. The average analyses of blood of twenty or more separate pooled samples are recorded in all tables.

The next step was to find which blood constituents gave the best index of the effect of estrogens, since the number of analyses that could be made for separate compounds was limited by the necessity of following the effects of many types of synthetic estrogens on the blood of many hundreds of individual birds. Therefore rather complete

analyses were made of blood samples from 12-week-old fryers receiving the basal ration as a control and the basal ration supplemented with the indicated amount of dianisylhexane, dianisylhexene, dianisylhexene plus lecithin, and lecithin alone. An inspection of the results listed in Table 2 indicated that two constituents, namely free cholesterol and total fatty acids, made significant changes under

TABLE 2. BLOOD ANALYSES OF NORMAL AND ESTROGEN FED TWELVE-WEEK-OLD FRYERS OF BOTH SEXES

Date	Feed	Whole blood total P	Plasma			Whole blood fatty acid	Cholesterol	
			Total P	Inorganic P	Lipoid P		Whole blood free	Total
		(mg. per 100 ml. blood)						
4–23	Control	112	25	5.4	8.4	443	50	105
	40 mg. dianisylhexane/lb. ration	111	28	6.1	12.0	473	49	116
	50 mg. dianisylhexene/lb. ration	112	35	6.2	14.0	765	52	113
4–30	Control	116	15	2.4	9.8	325	42	100
	40 mg. dianisylhexane/lb. ration	139	83	4.2	44.0	2587	214	322
	50 mg. dianisylhexene/lb. ration	170	100	5.6	60.0	3622	305	324
5–6	Control	126	29	5.8	16.6	399	70	160
	40 mg. dianisylhexane/lb. ration	174	135	14.5	39.6	1582	202	342
	50 mg. dianisylhexene/lb. ration	142	155	15.2	60.0	2276	392	438
5–7	Control +1% lecithin	106	26	5.1	10.0	308	62	93
	50 mg. dianisylhexene/lb. ration	142	51	7.8	28.0	765	83	148
	50 mg. dianisylhexene +1% lecithin/lb. ration	204	54	7.6	29.0	570	73	159
5–14	Control +1% lecithin	134	35	6.1	13.0	304	70	96
	50 mg. dianisylhexene/lb. ration	238	166	15.2	72.0	1208	305	410
	50 mg. dianisylhexene +1% lecithin/lb. ration	189	141	13.2	72.0	2321	268	386
5–24	Control +1% lecithin		35		17.0	402	65	127
	50 mg. dianisylhexene/lb. ration		250		62.0	3104	139	439
	50 mg. dianisylhexene +1% lecithin/lb. ration		189		62.0	4685	225	515
5–25[1]	Control +1% lecithin					635	47	
	50 mg. dianisylhexene/lb. ration					4952	227	
	50 mg. dianisylhexene +1% lecithin/lb. ration					9757	145	
5–27	Control +1% lecithin					340	57	
	50 mg. dianisylhexene/lb. ration					3548	165	
	50 mg. dianisylhexene +1% lecithin/lb. ration					3681	174	
5–28	Control +1% lecithin					281	52	
	50 mg. dianisylhexene/lb. ration					532	47	
	50 mg. dianisylhexene +1% lecithin/lb. ration					295	151	
6–1	Control +1% lecithin					369	57	
	50 mg. dianisylhexene/lb. ration					369	59	
	50 mg. dianisylhexene +1% lecithin/lb. ration					369	64	

[1] Estrogens discontinued after this date.

these conditions. Therefore only the free cholesterol and total fatty acids resulting from the use of the estrogens are reported after this date. The data in the second section of Table 2 were obtained to determine the use of lecithin as a carrier for the estrogens as well as to determine the duration of these chemical changes after discontinuing the estrogen feeding.

Table 3-A records the average free cholesterol and total fatty acid content of the blood of twelve-week-old fryers of mixed sex which had

been fed the control ration supplemented with various estrogens. Lecithin was added to certain of these rations to determine its effect on the utilization of the estrogens. The total fatty acid values in Table

TABLE 3. FREE CHOLESTEROL AND TOTAL FATTY ACID CONTENT OF BLOOD

A. 12-week-old fryers of both sexes

Free cholesterol							Total fatty acids						
3-18	3-20	3-22	3-24	3-26	3-28	3-30	3-18	3-20	3-22	3-24	3-26	3-28	3-30
(mg./100 ml. blood)							(mg./100 ml. blood)						
Control													
64.1	64.0	65.6	66.8	62.9	70.0	76.8	299	322	331	364	356	295	304
100 mg. triphenylchloroethylene/lb. ration													
57.0	62.9	81.7	86.7	107.0	222.0	263.0	251	710	1654	1357	3420	3431	3251
200 mg. triphenylchloroethylene/lb. ration													
59.8	93.5	110.2	120.6	138.5	213.0	278.0	269	680	1774	1895	3731	3226	4626
300 mg. triphenylchloroethylene/lb. ration													
62.9	102.1	119.0	173.0	183.3	256.0	263.0	312	562	1237	3352	3278	4332	5103
40 mg. dienestrol diacetate/lb. ration													
62.2	69.1	63.8	63.6	69.7	62.2	67.0	303	281	310	300	404	352	266
60 mg. dienestrol diacetate/lb. ration													
63.9	62.2	62.7	66.4	68.0	71.3	71.4	325	—	285	308	359	305	229
10 mg. dianisylhexene/lb. ration													
70.9	70.4	67.5	79.4	68.8	73.5	83.3	387	319	373	370	334	234	275
30 mg. dianisylhexene/lb. ration													
65.8	64.1	110.0	63.3	—	178.0	111.0	328	417	1508	482	450	1669	923
40 mg. dianisylhexene/lb. ration													
71.4	71.9	88.5	110.3	120.0	164.0	180.0	340	377	679	1118	1443	2323	2874
50 mg. dianisylhexene/lb. ration													
66.0	78.3	89.0	122.1	192.0	310.0	350.0	236	768	875	1361	2088	2486	3957
50 mg. dianisylhexene/lb. ration													
61.3	88.5	128.0	148.0	173.0	278.0	345.0	219	1319	2819	6016	8350	9530	9468
Ration plus 1% lecithin													
69.2	65.0	73.4	78.3	50.1	98.1	92.6	243	231	279	325	251	241	294

B. 12-week-old chicken fryers

	Total fatty acids				
	10-10	10-12	10-14	10-17	10-21
	(mg./100 ml. blood)				
Control	286	244	274	353	370
50 mg. dianisylhexene/lb. ration	222	816	1135	4043	7316
50 mg. dianisylhexene/lb. ration plus 5 grams soybean lecithin	275	512	1637	3521	5376
100 mg. triphenylchloroethylene/lb. ration	265	338	836	1994	3074
100 mg. triphenylchloroethylene/lb. ration plus 5 grams soybean lecithin	272	763	983	3077	3062

C. mature market hens

Free cholesterol			Total fatty acids		
12-10	12-16	1-4	12-10	12-16	1-4
(mg./100 ml. blood)			(mg./100 ml. blood)		
Control					
60.1	60.3	59.1	810	702	704
50 mg. dianisylhexene/lb. ration					
62.7	131.8	130.5	718	1820	4974

D. old cocks

Free cholesterol					Total fatty acids				
5-4	5-5	5-6	5-8	5-14	5-4	5-5	5-6	5-8	5-14
(mg./100 ml. blood)					(mg./100 ml. blood)				
Basal plus 1% lecithin									
93.5	120.0	110.0	87.7	96.2	217	226	226	353	522
15 mg. diethylstilbestrol injected in neck in oil solution May 4 plus 50 mg. dianisylhexene/lb. ration									
86.2	95.2	175.0	200.0	286.0	328	216	1229	2405	7392
50 mg. dianisylhexene/lb. ration									
96.2	90.1	106.0	100.0	217.0	208	276	393	384	2376

3-B measure the relative effect of soybean oil and a combination of soybean oil and soybean lecithin on the utilization of triphenylchloroethylene and dianisylhexene. Table 3-C gives blood data for mature market hens and Table 3-D for old cocks.

To compare the relative efficiency of implanted pellets and orally administered estrogen, young fryers were divided into three lots and fed as follows: One lot received the unsupplenented basal ration, the second lot received implanted pellets containing 15 mg. diethylstilbesterol each, and the third lot received dianisylhexene mixed with the feed at a dosage level of 50 mg. per pound of feed. These rations were fed for a period of 34 days after which the estrogen supplements

TABLE 4. ANALYSES OF BLOOD OF TWELVE-WEEK-OLD FRYERS RECEIVING ESTROGENS ADMINISTERED AS PELLET IMPLANTS AND AS A COMPONENT OF THE FEED

Feed	5-30	6-1	6-3	6-6	6-13	6-20	6-27*	7-2	7-5	7-7	7-9
	(mg./100 ml. whole blood)										
	Free Cholesterol										
Basal Ration (Control)	79	75	71	80	105	106	85	121	74	93	82
Diethylstilbestrol pellet implants in neck	77	78	72	87	109	128	76	86	61	68	82
50 mg. dianisylhexene/lb. ration	71	79	93	294	444	506	407	328	281	123	93
	Total Fatty Acids										
Basal Ration (Control)	388	355	295	369	355	340	455	366	322	195	279
Stilbestrol pellet implants in neck	369	413	369	413	399	370	277	674	277	274	224
50 mg. dianisylhexene/lb. ration	325	606	1493	4376	2833	5544	2212	2212	2475	374	250

* Estrogens discontinued after this date.

TABLE 5. ANALYSES OF DEHYDRATED FAT-FREE CHICKEN BONES AT END OF 17-DAY FEEDING PERIOD

Feed	Total Ash	Ca	P	Magnesium
	%	%	%	%
Basal	55.0	38.4	17.4	.483
Basal + 1% lecithin	50.8	31.0	16.7	.447
40 mg. dianisylhexene/lb. ration	55.0	38.3	17.6	.605
60 mg. dianisylhexene/lb. ration	53.0	36.2	17.1	.487
50 mg. dianisylhexene/lb. ration plus 1% lecithin	48.6	36.8	17.0	.472

were discontinued and all lots were fed the unsupplemented basal ration for an additional 7 days. The resulting analyses recorded in Table 4 indicate that in this determination the implanted pellet was utilized very slowly and much less effectively than a subcutaneous injection of an oil solution of the estrogen or the use of a mixed feed containing an oil solution of the estrogen. These results also indicate that the estrogen apparently is not stored in the body for any considerable period of time after estrogen feeding is discontinued.

During the progress of this investigation it was observed that the incidence of broken bones was greater in the estrogen-fed lots. Therefore the bone ash of the fat-free dehydrated bones of these chickens was determined on several occasions. The results on such series are recorded in Table 5 together with the calcium, phosphorus, and magnesium percentages of the ash.

To further test the possible changes in the ash content over a longer period of time the ash of bones from chickens receiving 50 mg. of dianisylhexene was determined each week for a five-week period, with the following percentages: 49.88, 49.44, 47.71, 47.90, and 48.70, respectively.

Turkeys

The effect of orally administered estrogens upon the total fatty acid and free cholesterol levels of turkey blood was studied in a similar

TABLE 6. CHOLESTEROL AND FATTY ACID CONTENT OF TURKEY BLOOD

Feed	Free cholesterol					Total fatty acids				
	1st	8th	15th	22nd	29th	1st	8th	15th	22nd	29th
	(mg./100 ml. blood)									
A. Twelve-week-old										
Basal Ration	65	75	103			276	306	309		
50 mg. dianisylhexene/lb. ration	71	226	206			275	1206	5603		
B. Twenty-week-old										
Basal Ration	81	99	95	102	81	365	450	517	383	440
40 mg. dianisylhexene/lb. ration	71	260	281	342	415	308	1987	4801	6380	6836
60 mg. dianisylhexene/lb. ration	73	253	309	309	501	361	3156	5271	7178	9135
80 mg. dianisylhexene/lb. ration	78	253	303	308	503	346	4261	6325	8348	9150

TABLE 7. TOTAL FATTY ACID IN BLOOD OF 12-WEEK-OLD TURKEYS AFTER 3 WEEKS ON FEED

	Basal Ration	50 mg. dianisyl-hexene per lb. feed	100 mg. triphenyl-chloroethylene per lb. feed	200 mg. triphenyl-chloroethylene per lb. feed
mg./100 ml. blood	406	5703	1409	3702

manner. The results recorded here are the average analyses of the blood of young turkeys ranging in age from 9 to 24 weeks. Table 6-A records the free cholesterol and total fatty acid values of the blood of two lots of turkey broilers which were 10 and 11 weeks of age at the beginning of the three-week premarket fattening period. One lot was fed the unsupplemented basal ration and the other the basal ration supplemented with estrogen as indicated. Table 6-B records both the free cholesterol and total fatty acids levels of the blood of 20-week-old turkeys fed different levels of dianisylhexene during a four-week feeding period. Table 7 records the average total fatty acid content of the blood of 12-week-old turkey broilers which had been fed one level of dianisylhexene and two levels of triphenylcholoroethylene during a three-week fattening period.

SUMMARY

Oral administration of estrogens was more effective with poultry than pellet implants. Of the blood components the fatty acid, choles-

terol and lipoid phosphorus showed phenomenal changes following the administration of estrogens. Injections of oil solutions of estrogens produced chemical changes within a few hours. The maximum change with either pellet implant or oral administration was indicated within a week. Blood constituents returned to near normal within 3 to 4 days after estrogen administration was discontinued. Although the bones of these fowl seemed to be more fragile, there was no significant change in tibia ash analyses.

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AN ATTEMPT TO EXPLAIN THE ANOMALOUS ACTION OF LUGOL'S SOLUTION IN EXOPHTHALMIC GOITER[1]

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In a series of investigations extending over a number of years we have followed a certain working hypothesis, although we realize that other hypotheses have been followed by other investigators. We consider a goiter a thyroid gland enlarged by compensatory hypertrophy and therefore capable of producing more thyroglobulin than the normal thyroid. The distinction of diffuse and adenomatous goiter often fails due to the finding of minute or hidden adenomas in goiters previously classified as diffuse. Kocher, of Berne, Switzerland, would not allow iodine used on his goiter patients for fear of making them all hyperthyroid but Plummer, of the Mayo Clinic, dosed his patients with Lugol's solution and demonstrated its anomalous action in lowering the basal metabolic rate in exophthalmic goiter.

We have shown that thyroglobulin from untreated goiters has a low thyroxine content (Cavett, Rice and McClendon, '35). One of these thyroglobulins from a goiter contained only 0.0046% thyroxine whereas thyroglobulin from as nearly normal thyroids as we could obtain at autopsy contained about 0.3% thyroxine. When these thyroglobulins were fed to rats, at the dose of 280 micrograms of thyroxine iodine per 100 sq. cm. of body surface, they increased the metabolic rate in proportion to their thyroxine content (McClendon, Foster and Cavett, '41). The presence of live thyroid tissue is not necessary in iodinating thyroglobulin. When one of these goitrous thyroglobulins was dissolved in physiological salt solution and stirred with iodine at body temperature and *p*H for 20 hours its thyroxine iodine content was increased from 0.06% to 0.342% (McClendon and Foster, '44, a). Its effect on the metabolic rate of rats was also increased (McClendon and Foster, '44, b). We must conclude, therefore, that there is not enough elemental iodine in a goiter to fully iodinate the thyroglobulin, and that the increased metabolic rate in exophthalmic goiter is due to release of *large amounts* of thyroglobulin whose *total* thyroxine content is greater than normal.

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[1] We are indebted to the Lederle Laboratories for the thiouracil (deracil) fed to the sheep.

Since the action of Lugol's solution is to raise the thyroxine content of goitrous thyroglobulin (Cavett, Rice and McClendon, '35) its action in lowering the basal metabolic rate must be sought outside the thyroid, perhaps in the anterior pituitary. We have not attempted to determine the thyroxine content of the anterior pituitary, but since thyroxine seems to be present chiefly in protein combination in the body we determined the protein bound iodine in the pituitary.

It is well known that an increase in protein bound iodine *arising from the thyroid* will inhibit the production of thyrotropin by the pituitary. It seems reasonable that this protein bound iodine must reach the pituitary in order to have its effect. If protein bound iodine were *synthesized within the pituitary itself*, how could the pituitary distinguish between that and the supply from the thyroid gland? Perhaps the protein bound iodine synthesized within the pituitary might inhibit the pituitary's secretion of thyrotropin. Perhaps the protein bound iodine synthesized within the pituitary would not diffuse out and certainly the determinations on sheep given below suggests that it accumulates there. If this were true it would explain the effect of Lugol's solution in inhibiting the thyroid. The Lugol's solution would first iodinate protein in the pituitary, this protein would inhibit the thyrotropin production. The lessened amount of thyrotropin would abolish the stimulating effect on the thyroid, and the resting thyroid would not release thyroglobulin into the circulation, but we wished to put this hypothesis to the test. Due to the great variability in the diet of dogs we decided to use a couple of sheep (ewes) which a farmer assured us were twins and had always grazed in the same pasture. Although these sheep were 7 years old one could not be out of sight of the other without baaing continuously. One of these sheep was fed 100 milligrams potassium iodide a day in the form of a sandwhich. Some of the iodide changed to iodine and colored the starch of the bread blue. The other was given 3 grams of thiouracil a day in the form of a sandwich, but refused it from the third day onward so it was given by means of a tube and plunger. The pituitary of the sheep fed 100 mg. potassium iodide a day for 66 days contained 263 per cent as much protein bound iodine as the pituitary of the sheep receiving 3 grams a day of thiouracil (deracil) for 41 days (McClendon and Foster, '47). The weight of the potassium iodide fed pituitary was 1.625 grams and that of the thiouracil fed 1.18 grams. Due to the expense of sheep we decided to use chickens for continuing these experiments.

MATERIAL AND METHODS

More than 200 New Hampshire Red chickens, 12 weeks old and weighing about 4 pounds each were used. Seventy-one were used for controls, 74 were given 4 cc. of Lugol's solution per gallon of drinking water and 71 were fed 0.2% thiouracil in their feed to deplete their bodies of elemental iodine. Two experiments were run, one of 2 weeks and one of 3 weeks. At the end of these

experiments the chickens were killed, weighed, and pituitary and thyroid glands removed, weighed, and after the inorganic iodine was extracted, preserved in vials for protein bound iodine analysis.

The thyroid glands are paired and lie in the thorax, mesially to the jugular vein, in the angle formed by the subclavian and common carotid arteries. They were removed after first removing the crop. The pituitaries were removed by operating from the hard palate after removal of the lower jaw.

The inorganic iodine was extracted 5 minutes with cold methanol and 5 minutes with cold acetone in the 3 week experiments, but for fear that this was not sufficient it was extracted an additional 2 months with cold acetone

TABLE 1. MEAN VALUES OF PROTEIN BOUND IODINE IN PITUITARY AND THYROID OF CHICKS FED 0.2% THIOURACIL

Weeks of feeding	Number of chicks	Body wt. lbs.	Pituitary						Thyroid		
			Wt. mg.	S.D.	St. error of mean	Prob. error of mean	Iodine		Milligrams		% Iodine dry
							γ	γ per gm.	wet	dry	
3	12	3.38	15.5				0.041	2.63	518	87	0.025
3	12	3.47	13.5				0.038	2.78	530	89	0.024
3	24	3.42	14.5	3.39	0.69	0.47	0.039	2.70	524	88	0.025
2	10	4.09	19.5				0.032	1.64	437	80	0.018
2	10	4.24	21.0				0.062	2.96	452	98	0.021
2	10	3.49	17.1				0.048	2.81	360	75	0.019
2	10	4.04	14.6				0.037	2.54	393	66	0.020
2	10	3.95	20.9				0.051	2.44	437	99	0.017
2	50	3.96	18.6	5.58	0.79	0.53	0.046	2.47	416	83	0.019
2 and 3	74	3.79	17.3				0.044	2.53	451	85	0.021

TABLE 2. MEAN VALUES OF PROTEIN BOUND IODINE IN PITUITARY AND THYROID OF CONTROL CHICKS

Weeks of feeding	Number of chicks	Body wt. lbs.	Pituitary						Thyroid		
			Wt. mg.	S.D.	St. error of mean	Prob. error of mean	Iodine		Milligrams		% Iodine dry
							γ	γ per gm.	wet	dry	
3	12	3.70	18.4				0.048	2.63	154	37	0.175
3	10	3.89	17.7				0.048	2.71	144	32	0.257
3	22	3.79	18.1	6.33	1.35	0.91	0.048	2.67	149	35	0.216
2	10	4?	15.3				0.054	3.52	171	55	0.179
2	10	4?	14.6				0.042	2.87	181	51	0.199
2	10	4?	15.6				0.066	4.24	188	51	0.215
2	10	4?	19.9				0.056	2.81	172	48	0.258
2	9	4?	19.7				0.051	2.60	199	54	0.219
2	49	4?	17.0	5.00	0.71	0.48	0.054	3.17	182	52	0.214
2 and 3	71	4?	17.3				0.052	3.01	172	47	0.215

in the 2 week experiments. The remainder was considered protein bound iodine (McClendon and Foster, '44, c). The 2 month extraction showed no lower values of protein bound iodine except in those chickens fed Lugol's solution for only 2 weeks. But one would expect a less effect of Lugol's solution in 2 weeks than in 3 weeks. Therefore we believe that the extraction of inorganic iodine was complete within the limit of error of the methods. The iodine determinations were made by the method of McClendon and Bratton ('38).

RESULTS

From Tables 1, 2 and 3 it may be seen that thiouracil markedly increased both wet and dry weight and lowered the protein bound iodine in the thyroid to 10 per cent of its normal value, whereas Lugol's solution did little to the wet and less to the dry weight but more than doubled the protein bound iodine in the thyroid. The effect on the pituitary is much less and therefore statistical methods were applied to it. It was thought that for strict application of statistics the 3 week and 2 week experiments should be treated separately.

In the 3 week experiments the mean pituitary weight in the thio-

TABLE 3. MEAN VALUES OF PROTEIN BOUND IODINE IN PITUITARY AND THYROID OF CHICKS DRINKING 4 CC. LUGOL'S PER GAL.

Weeks of feeding	Number of chicks	Body wt. lbs.	Pituitary						Thyroid		
			mg. Wt.	S.D.	St. error of mean	Prob. error of mean	Iodine		Milligrams		% Iodine dry
							γ	γ per gm.	wet	dry	
3	10	3.79	16.8				0.075	4.45	220	60	0.682
3	11	3.53	17.3				0.063	3.63	177	49	0.660
3	21	3.65	17.1	5.77	1.26	0.85	0.069	4.01	198	54	0.671
2	10	4.04	22.7				0.075	3.31	220	62	
2	10	3.94	26.4				0.104	3.94	196	48	0.724
2	9	3.53	18.3				0.120	6.56	186	47	0.325
2	10	3.79	20.2				0.062	3.07	234	62	0.317
2	11	3.84	18.9				0.045	2.40	217	55	0.306
2	50	3.83	21.3	7.49	1.06	0.71	0.080	3.75	209	55	0.418
2 and 3	71	3.78	20.1				0.077	3.81	204	55	0.502

uracil fed chickens is 14.49 ± 0.47 mg. whereas that of the controls is 18.05 ± 0.91 mg. The standard error of the difference of these two means is 2.347, and the probability of this not being due to chance is 50 to 1. The mean weight of the pituitary in the Lugol's fed chickens is 17.08 ± 0.85 and the standard error of the difference from the control is 0.45. The probability of this difference being due to chance is about 1 to 1 and therefore it is doubtful that Lugol's solution affected the weight of the pituitary.

In the 2 week experiments the mean weight of pituitary of the thiouracil fed chickens is 18.61 ± 0.53 mg. and that of the controls 16.96 ± 0.48 mg. The standard error of the difference of these means is 1.55. The probability of this not being due to chance is about 7 to 1 but the difference is in opposite direction to the 3 week experiment. It therefore seems doubtful that thiouracil had any effect on the weight of the pituitary. The mean pituitary weight of the Lugol's fed chickens is 21.31 ± 0.71 and the standard error of the difference between this and the control is 1.7 and the probability of this difference not being due to chance is about 1483 to 1. But since there was no difference in the feeding of the control group in the 2 and 3 week experiments it is per-

missible to compare the 2 week Lugol's fed chickens with the 3 week controls in which case the standard error of the difference of means is 1.71 and the probability of the difference not being due to chance is 16 to 1. Whereas the data suggest that Lugol's solution increases the weight of the pituitary gland they do not prove the point.

Although the glands of the 216 chickens were weighed individually the protein bound iodine in a single pituitary was about 0.05 microgram and too small for accurate determination. Therefore the mean value of about 10 glands was determined by analyzing the group and dividing by the number of glands. The number of groups is not sufficient for statistical analysis but the large differences as shown in

TABLE 4. MEAN VALUES OF PROTEIN BOUND IODINE IN THE PITUITARY GLANDS OF NORMAL CHICKENS COMPARED WITH THOSE GIVEN THIOURACIL OR LUGOL'S SOLUTION

Treatment	Weeks of exp.	No. of chicks	γ	γ per gm.	Per cent of control	
					γ	γ per gm.
Thiouracil	3	24	0.039	2.70	75	90
	2	50	0.046	2.47	87	82
	2 and 3	74	0.044	2.53	85	84
Control		71	0.052	3.01		
Lugol's	3	21	0.069	4.01	133	133
	2	50	0.080	3.75	154	125
	2 and 3	71	0.077	3.81	148	127

Table 4 are impressive. Since there was no difference in the feeding of the controls in 2 as compared with the 3 week experiments they have been combined.

Thiouracil reduced the protein bound iodine in the pituitary gland to 85 per cent of its normal value and Lugol's solution increased it to 148 per cent of normal. If we take into consideration the weight of the pituitary gland and calculate the micrograms of protein bound iodine per gram of gland, thiouracil reduced it to 84 per cent of normal and Lugol's solution increased it to 127 per cent of normal.

We may conclude that Lugol's solution increases the protein bound iodine of the pituitary gland and that is a possible explanation of its action in exophthalmic goiter. For this to be true it is not necessary that Lugol's solution should always lower the basal metabolic rate, but only in those cases where a high rate is due to a high liberation of thyrotropin by the pituitary gland.

That there may be other mechanisms of the action of Lugol's solution is freely admitted, but they have been reviewed by Rawson ('47) and others and space does not admit of their adequate evaluation.

SUMMARY

The thyroid and pituitary glands of 216 twelve week old chickens were weighed and analyzed for protein bound iodine. One third had been given 0.2 per cent thiouracil in the feed, one third had 4 cc. of Lugol's solution added per gallon of drinking water and one third were used as controls.

Thiouracil markedly increased both wet and dry weight of the thyroid glands and reduced the protein bound iodine to 10 per cent of its normal value. The protein bound iodine of the pituitary glands was reduced to 85 per cent of its normal value.

Lugol's solution increased the protein bound iodine of the thyroid 133 per cent and of the pituitary 48 per cent. It seems probable that local action of this protein bound iodine in the pituitary might inhibit the release of thyrotropin and explain the anomalous action of Lugol's solution in exophthalmic goiter.

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METABOLISM OF THE STEROID HORMONES: THE ISOLATION OF ETIOCHOLANEDIOL-3(α), 17(α) FROM HUMAN URINE[1]

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ETIOCHOLANEDIOL-3(α), 17(α) has been isolated from the urine of two patients. One patient was a male with Addison's disease (Patient A) who received 1350 mg. of dehydroisoandrosterone. The other patient was a young woman (Patient B) who had symptoms of virilism and a high excretion of androgens and 17-ketosteroids (Schiller *et al.*, 1945a).

No isolation of etiocholanediol-3(α), 17(α) has been reported at the present time. A small amount of a non-ketonic compound which appeared to be an impure sample of etiocholanediol-3(α), 17(α) was reported by Dorfman *et al.* (1939). Butenandt (1937) described the isolation of this diol from a urinary extract which had been reduced with aluminum amalgam. It was not found in urinary extracts not treated with aluminum amalgam.

EXPERIMENTAL

Patient A

The urine was collected for 3 days during oral administration of 450 mg. per day of dehydroisoandrosterone.[2] The non-ketonic digitonic soluble fraction was prepared from acid hydrolyzed urine by a method previously described (Schiller, Dorfman, and Miller, 1945). This fraction was acetylated and chromatographed on aluminum oxide (Merck-Brockmann). Five mg. of a compound (M.P. 124–127°C.) was obtained by elution with 50% benzene-petroleum ether. The melting point of this compound was not depressed when mixed with a sample of etiocholanediol-3(α), 17(α) diacetate, melting point 126–127°C. prepared by reduction of etiocholanol-3(α)-one-17 with sodium and isopropyl alcohol and subsequent acetylation.

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[2] The dehydroisoandrosterone was generously supplied by Ciba Pharmaceutical Products, Inc.

Patient B

The neutral non-ketonic digitonin non-precipitable fraction was prepared from a benzene extract of 74 liters of unhydrolyzed urine. The material was chromatographed 3 times on aluminium oxide. 3 mg. crystals m.p. 232–233°C. were obtained by elution with 0.1% absolute ethanol in benzene. The melting point was not depressed by mixture with etiocholanediol-3(α), 17(α) m.p. 232°–234° prepared from etiocholanol-3(α)-one-17. The material was acetylated yielding a product m.p. 125°–126°C. When mixed with etiocholanediol-3(α), 17(α), diacetate m.p. 126°–127° prepared from etiocholanol-3(α)-one-17, the mixture melted at 125°–127°.

The melting points[3] reported for etiocholanediol-3(α), 17(α) are 232°C. (Butenandt, Tscherning, and Dannenberg, 1937) and 236–236.5°C. (corr.) (Ruzicka, Goldberg, and Bosshard, 1937). Ercoli (1938) reported a melting point of 124.5–125.5°C. (corr.) for the diacetate.

DISCUSSION

Whether etiocholanediol-3(α), 17(α) arose as a metabolite of dehydroisoandrosterone cannot be decided at this time. The yield of the diol was exceedingly small. It is known, however, that dehydroisoandrosterone is metabolized to etiocholanol-3(α)-one-17 (Mason and Kepler, 1945; Miller, Dorfman, Miller). It is possible that the diol represents a further reduction of etiocholanol-3(α)-one-17.

The etiocholanediol-3(α), 17(α) was isolated from the extract prepared from the unhydrolyzed urine of the woman with symptoms of virilism. The unhydrolyzed urine extracts contained half of the 17-ketosteroids. It would be expected that the extract prepared from the residual urine after acid hydrolysis will contain additional quantities of the diol. This fraction has not been studied at the present time.

SUMMARY

Etiocholanediol-3(α), 17(α) has been isolated from the urine of a males with Addison's disease who received dehydroisoandrosterone and from the urine of a young woman with symptoms of virilism.

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[3] All melting points were taken with the Fischer-Johns apparatus and are uncorrected.

HYALURONIDASE LEVELS OF RAT TESTES AS RELATED TO AGE, HYPOPHYSECTOMY AND CRYPTORCHIDISM[1]

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SINCE the discovery of the enzyme hyaluronidase in mammalian testes, one of the problems in which we have been interested has been to determine by what means the enzyme level in the testes might be altered. Previous to identification of hyaluronidase with the "spreading factor," Sprunt, *et al.* (1939) showed that the latter was present in the testes of immature rabbits and cryptorchid rats in amounts less than in normal adults. In a preliminary note (Leonard and Perlman, 1946) it was reported that hyaluronidase, determined by the turbidimetric method, was lower in immature, cryptorchid, and hypophysectomized rats. The details of these experiments are reported here.

METHODS

Determinations of hyaluronidase were made according to the previously described turbidimetric method (Leonard, Perlman and Kurzrok, 1946). The units of enzyme are expressed as turbidity reducing units (TRU). The same batch of hyaluronic acid was used throughout these experiments. Assays also were made using a modification of the rat-ova test (Leonard and Kurzrok, 1946) in which a comparison of the time required to disperse the follicle cells as related to dosage was used as a measure of relative activity. Testes homogenates were prepared in acetate buffer (pH 6) for the turbidimetric tests and in Ringer's solution when the rat-ova test was used. Testes of several rats were pooled in making certain of the extracts in order to obtain sufficient material for assay. Histological examination of the testes from rats were made on samples representative of the several experiments.

RESULTS

Experiments were performed to determine the relationship of age and testes weight to the enzyme level. By the turbidimetric method it was found that traces of enzyme up to 2 TRU were present in testes of rats 21–30 days old and with increasing age there was an increase up to 10 TRU per gram of tissue (Table 1). Because the variation in size of the rats from different litters at any given age would be reflected in

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the size of the testes, it seemed best to correlate testis weight with enzyme level. Since all testes extracts were prepared so that 1 gram of tissue was equivalent to 1 cc. of extract, values of less than 2 units could not be obtained, although in these instances traces of activity were observed. No attempt was made to concentrate the enzyme as it seemed sufficient to show that a decreased amount of hyaluronidase was present in the small testes of the youngest rats. In order to prove that the traces of activity were real and not an artifact, aliquot samples were heated to destroy the enzyme and in every case assay of the heated material indicated no activity.

The results of similar assays but using the dispersal time of the

TABLE 1. HYALURONIDASE ACTIVITY OF TESTES AS A FUNCTION OF AGE AND TESTIS WEIGHT IN THE RAT

Age group (days)	No. of assays	No. of rats	Testes weight per rat (gm.)	TRU per gram of testis
90 and over	7	15	1.80–3.35	10–11
50	4	6	1.40–1.56	10
32–44	3	13	0.67–1.12	5–7
21–30	3	14	0.42–0.45	<2–2

follicle cells indicated the same trend in the appearance of the enzyme with increasing development of the testes (Table 3). The concentration of enzyme was too low to be detected in the 26-day old rats and had about reached its adult level in 50-day old rats.

Histological examination of the testes revealed only the early stages of spermatogenesis in the 26-day old rats. Those at 34 days showed an increase in tubule size but no marked difference in the stages of spermatogenesis. At 50 days the tubules were still larger, and possessed spermatozoa as well as spermatids in great numbers.

In another experiment adult rats, weighing not less than 250 grams, were made cryptorchid by severing the gubernaculum and anchoring the testes to the abdominal wall. Assay of the enzyme concentration five days later showed no decrease in the hyaluronidase level but by the 12th day a low concentration was found beyond which no further drop occurred on either 21 or 30 days (Table 2). The rat-ova test indicated a slight drop in enzyme by 10 days and no detectable enzyme on the 21st day (Table 3).

Histologically, the testes of 5-day cryptorchid rats revealed slightly smaller tubules, loss of many sperm, and some degenerating epithelium. By the 12th day the tubules showed a characteristic marked degeneration, the germinal epithelium in some tubules, however, was intact although no fully formed sperm were present. No further degenerative changes were observed in the testes of rats on the 21st day after operation.

TABLE 2. HYALURONIDASE ACTIVITY IN RAT TESTES FOLLOWING CRYPTORCHIDISM AND HYPOPHYSECTOMY

Exper.	No. of assays	No. of rats	Testes weight per rat (gm.)	TRU per gram of testes
Normal adults	7	15	1.80–3.35	10–11
Cryptorchid (days)				
5	1	4	2.20	11
12	1	3	1.50	3
21	1	2	1.40	4
30	2	4	1.2–1.6	3
Hypophysectomy (days)				
10	9	15	1.6–2.5	11.7 (10–15)
20	3	6	1.0–1.2	8.3 (7–10)
24	5	9	0.7–0.8	3.8 (1–6)
29	1	1	0.40	4
30	1	1	0.40	4
62	1	7	0.34	3

Adult rats over 250 grams body weight were hypophysectomized and the testes assayed at intervals from 10 to 62 days later. It was noted that the fall in enzyme level was slight up to 20 days post-operatively but dropped rapidly by 24 days (Table 2). Beyond this the enzyme level constantly remained low. The results from the rat-ova test were similar in that only a very small drop in enzyme level

TABLE 3. HYALURONIDASE FROM RAT TESTES UNDER DIFFERENT EXPERIMENTAL CONDITIONS ASSAYED BY THE RAT-OVA METHOD

Exper.	Testes weight per rat (gm.)	Testes extract (gm. equiv.)	Time of follicle cell dispersal (hours)
Normal Adults	2–3.2	.05–.10	$\frac{3}{4}$–1
26 days	0.4	0.20	0 dispersal
26 days	0.4	0.20	0 dispersal
34 days	0.7	0.20	2; $2\frac{1}{4}$; $2\frac{1}{4}$
50 days	1.6	0.20	$1\frac{1}{2}$; $1\frac{1}{2}$
50 days	1.4	0.20	$\frac{1}{2}$, $\frac{1}{2}$
		0.10	$\frac{3}{4}$, $\frac{3}{4}$
Cryptorchid			
10 days		0.20	$\frac{1}{2}$; $\frac{3}{4}$
		0.10	1; $1\frac{1}{2}$
21 days		0.20	0 dispersal
Hypophysectomy			
10 days		0.20	$\frac{1}{2}$; $\frac{3}{4}$
		0.10	1; $1\frac{1}{2}$
20 days		0.20	$\frac{1}{2}$; $\frac{1}{2}$
		0.10	$\frac{3}{4}$; $\frac{3}{4}$
24 days		0.20	$4\frac{1}{4}$; $3\frac{1}{2}$
24 days		0.20	0 dispersal

was obtained up to the 20th day but a marked drop was observed by the 24th day.

Histologically, the testis tubules of 10-day hypophysectomized rats showed relatively little change. Many sperm and spermatids were present, although the tubules appeared smaller. By the 20th day the tubules contained many secondary spermatocytes, only a few spermatids, and no sperm. Testes examined on the 24th day showed no spermatids, and marked reduction in the depth of the germinal epithelium.

DISCUSSION

These results indicate that some enzyme appears in the germinal cells of the testes in the absence of fully developed sperm. This was seen in the testis of immature, cryptorchid, and hypophysectomized rats. In the last two conditions some germinal elements were seen in a number of the tubules although widespread injury was observed in most of them. These remaining cells might account for the trace of activity observed.

Where normal and presumably maximum numbers of spermatozoa were present in the testes, the maximum amount of hyaluronidase was obtained. This might be attributed to a higher content of enzyme in the cells or to the greater proportion of enzyme-producing cells to the non-germinal elements in the gonads. It seems unlikely that other cells would produce the enzyme. For example, the interstitial cells of cryptorchid testes were normal and those of the hypophysectomized rat were atrophied yet the enzyme concentration was approximately the same. It is conceivable, however, that the Sertoli cells might also contribute some enzyme.

Sprunt, *et al.* (1939) showed that the spreading factor was decreased in the testes of immature rabbits and cryptorchid rats. They stated that the amount of spreading factor is related to the amount of spermatogenesis. Since it has been shown that testes spreading factor and hyaluronidase are the same or closely related substances our results confirm those of the above investigators. Swyer (1947) however was unable to demonstrate any hyaluronidase in the testes of cryptorchid or prepubertal rabbits.

Our original problem was to determine what factors might affect the level of hyaluronidase in the cells producing the enzyme and it seemed that the usual metabolic derangements accompanying hypophysectomy would affect the metabolism of these cells and thereby the production of the enzyme. However, the failure to obtain any decrease in enzyme 10 days after hypophysectomy and only slightly at 20 days seems to indicate that alteration of the enzyme level in the testes without severe injury to the germinal epithelium will be very difficult if not impossible.

SUMMARY

Hyaluronidase determinations were made on rat testes by the turbidimetric method and the rat-ova test in animals of varying ages, and following cryptorchidism and hypophysectomy. A gradual increase in the amount of enzyme per gram of tissue was noted between 21 days of age and sexual maturity. After experimental cryptorchidism, the hyaluronidase level was low by the 10th day following the operation and after hypophysectomy it was low by the 24th day. Enzyme levels were correlated directly with the degree of development of the germinal epithelium. All of the enzyme of the testes was not accounted for by mature sperm since it was detected in testes devoid of sperm.

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THE PATHOLOGY OF THE ADRENAL GLAND IN ADDISON'S DISEASE WITH SPECIAL REFERENCE TO ADRENOCORTICAL CONTRACTION

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"Cytotoxic" necrosis, atrophy or contraction of the adrenal cortex associated with functional insufficiency has been recognized as a clinicopathologic entity for many years (Barker, 1929; Guttman, 1930; Duffin, 1943). It is also well known that the incidence of adrenocortical contraction as a cause of Addison's disease now equals or exceeds that of tuberculosis of the adrenal. Nevertheless, the continuing uncertainty among endocrinologists about the essential nature of the process is evidenced by a recent report (Rogoff, 1944) in which the lesions of contraction were confused with ischemic lesions of the adrenal.

This study was stimulated by the receipt at the Army Institute of Pathology, from the beginning of World War II through 1946, of pathologic material from 15 patients who exhibited adrenocortical contraction at autopsy; during the same period only 10 cases of Addison's disease resulting from tuberculosis or fibrocaseous destruction were accessioned. The time at which the first oubtreak of epidemic hepatitis reached its height during the war coincided with the period during which many of the examples of adrenocortical contraction appeared. During a discussion, Dr. Balduin Lucké, who did extensive investigation on epidemic hepatitis, pointed out that the patterns of postnecrotic parenchymal collapse and secondary superimposed nodular "regeneration" and proliferation, which characterized the hepatic lesions, were duplicated in the adrenal. The similarity between adrenal contraction and certain hepatic processes had been noted before (Wells, 1930; Kiefer, 1927; Duffin, 1943).

Prior to World War II only 6 cases of adrenocortical contraction had been accessioned at the Institute, in contrast to 21 cases of Addison's disease caused by tuberculosis. The relative incidence of tuberculosis and contraction in the cases of Addisons' disease in the files of the Army Institute of Pathology is given by 5 year periods in Table 1. The apparent relative increase in the incidence of adrenocortical contraction has been pointed out many times in this country (Wells, Humphreys and Work, 1937; Duff and Bernstein, 1933) and in England (Hellier, 1930; Susman, 1930; Barnard, 1930), but it is

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not known whether the condition is really more frequent or whether the incidence of adrenal tuberculosis is decreasing.

The 21 cases of adrenocortical contraction (Table 2) include 17 in which there was bilateral contraction, 2 in which there was unilateral contraction and the opposite adrenal was not found at autopsy and 2 in which neither adrenal was found. Since it is well known that a de-

TABLE 1. RELATIVE INCIDENCE OF ADRENAL TUBERCULOSIS AND CONTRACTION IN 52 CASES OF ADDISON'S DISEASE IN THE FILES OF THE ARMY INSTITUTE OF PATHOLOGY

Period	No. of cases with Adrenal Tuberculosis	No. of cases with Adrenal Contraction
1915–20	2	1
1921–25	3	0
1926–30	7	3
1931–35	4	0
1936–40	5	2
1941–46	10	15
Total	31	21

formed, shrunken adrenal may be difficult to identify, inclusion of the 2 equivocal cases appears justified.

CLINICAL FEATURES

Significant clinical data in the 25 cases of the Institute series are listed in Table 2. All the patients were men; except for 1 Indian and 1 Negro, they were white.

Seven patients had known Addison's disease with all the typical clinical manifestations, and in 4 other instances this diagnosis had been considered. Seven patients received hormonal therapy. Some died during an infection or as the result of unusual strain, such as that occasioned by a trip away from the hospital. Others for whom the diagnosis of Addison's disease had been made or suspected died suddenly and unexpectedly before specific therapy was begun.

In 7 instances the clinical syndrome was so atypical and confusing and the course of the disease so short that the diagnosis of adrenal insufficiency was not entertained. Consequently, only 1 of the 5 patients who died within a day after hospitalization received hormonal therapy.

Nausea, anorexia, vomiting, diarrhea and abdominal pain are common symptoms of Addison's disease. Nevertheless, 2 patients had vomiting and diarrhea so extreme that they were believed to have been poisoned, and extensive toxicologic studies were carried out. Neuropsychiatric manifestations dominated the picture in 5 instances. Three patients were considered psychotic, 1 was thought to have suffered an intracranial hemorrhage and 1 was given the diagnosis of myasthenia gravis. One patient was operated on for supposed rup-

TABLE 2. CLINICAL DATA IN 25 CASES OF ADRENAL INSUFFICIENCY

Case no.	Patient's years	Clinical manifestations	Duration	Clinical diagnosis	Comment
		Bilateral Adrenocortical Contraction			
1	33	Gastrointestinal disturbances, hypotension, pigmentation, neuropsychiatriac disturbances, weakness	6 months	Psychosis; malnutrition; Addison's disease (?)	Received hormonal therapy; episode of weakness following pneumonia 2 years before fatal illness
2	30	Anginal seizures	1 day	Coronary occlusion	Pericarditis
3	18	Gastrointestinal disturbances, hypotension, pigmentation, weakness	1 month	Addison's disease (?)	—
4	32	Gastrointestinal disturbances, hypotension, pigmentation, weakness, neuropsychiatric disturbances	1 month	Psychosis; Addison's disease (?)	—
5	23	Weakness, hypotension, loss of weight	2 weeks	Myasthenia gravis; Addison's disease (?)	—
6	41	Semicoma, hypotension	1 day	Psychosis	Previous collapse after forced march
7	22	Gastrointestinal disturbances, hypotension, pigmentation, weakness	2 months	Addison's disease	Treated with sodium chloride, quinine, plasmochin; pericarditis
8	25	Hypotension, pigmentation, weakness, foot drop	7 months	Addison's disease	Received hormonal therapy; relapse during infection of respiratory tract
9	22	Abdominal crisis, pigmentation, electrocardiographic changes	1 day	Perforated peptic ulcer; coronary occlusion	Laparotomy; pericarditis
10	24	Coma, convulsions	1 day	Acute gastroenteritis; poisoning (?)	—
11	27	Gastrointestinal disturbances, hypotension, pigmentation, weakness	7 weeks	Addison's disease	Received hormonal therapy; relapse while out of hospital
12	22	Hypotension, pigmentation, weakness, gastrointestinal disturbances	9 months	Addison's disease	Previous psychotic episode; received hormonal therapy; relapse during infection of respiratory tract
13	23	Gastrointestinal disturbances, hypotension	1 week	Gastritis; poisoning (?); heat stroke (?)	—
14	38	Gastrointestinal disturbances, hypotension, pigmentation, weakness	6 months	Addison's disease	—
15	45	No data	—	—	Pigmentation & alopecia at autopsy
16	35	No data	—	—	Pigmentation & emaciation at autopsy
17	—	No data	—	—	Pigmentation & emaciation at autopsy
		Unilateral Adrenocortical Contraction (Other Adrenal Not Found)			
18	20	Infection of respiratory tract, gastrointestinal disturbances, pigmentation, coma	1 day	Addison's disease	Previous poisoning by carbon monoxide; received hormonal therapy
19	20	Gastrointestinal disturbances, weakness, hypotension	2 weeks	—	Previous thyrotoxicosis; thyroidectomy; received thyroid
		Neither Adrenal Found			
20	45	Coma	4 days	Intracranial accident	—
21	24	Hypotension, weakness, gastrointestinal disturbances, pigmentation	1 week	Addison's disease	Received hormonal therapy
		Acute Adrenal Lesions			
22	25	Gastrointestinal disturbances, hypotension, pigmentation, weakness, loss of weight	2 weeks	—	Previous malaria, dysentery; received hormonal therapy
23	42	Gastrointestinal disturbances, hypotension, weakness, emaciation	3 months	Malnutrition; psychosis	Purpura; nutritional anemia (?)
24	44	Gastrointestinal disturbances, hypotension, uremia	5 days	Nephritis	—
25	22	Gastrointestinal disturbances	1 day	Poisoning (?)	Had been drinking heavily for 2 weeks; found dead in bed

tured peptic ulcer. Two patients were believed to have had acute coronary occlusion; both had fibrinous pericarditis as well as adrenocortical contraction. It is worth pointing out that a third patient, with clinically typical Addison's disease, had fibrinous pericarditis at autopsy.

The diagnosis of Addison's disease in some of the cases with atypi-

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4

cal clinical pictures might be questioned. However, since adrenocortical contraction was the only significant lesion disclosed by autopsy, it seemed reasonable to ascribe death to adrenal insufficiency despite the absence of the full blown clinical syndrome. Furthermore, in some instances pigmentation, which had not been noted during life, came to light at autopsy; and in still others it was recalled in retrospect, after autopsy, that the patient had shown a remarkable response to saline infusions.

MORPHOLOGIC FEATURES

The adrenals were usually small and misshapen. The combined weights of the two glands were not infrequently as low as 2.5 Gm. The microscopic appearance of the contracted adrenals varied. In some cases simple collapse and shrinkage of the cortical parenchyma had taken place, so that the original outline of the gland was preserved (Fig. 2). In such cases the adrenal was made up almost entirely of medullary tissue, with only remnants of cortex (Fig. 5). The peripheral portions of the glands, where no medulla was present, were reduced to mere thin bands (Fig. 6). In the collapsed cortical portions the preexisting argyrophilic recticular framework was condensed (Fig. 7), but there was no productive or cicatricial fibrosis.

Residual groups of cortical cells persisted in places, and nodular proliferation (Fig. 3) sometimes obscured the over-all shrinkage. In 1 case hyperplastic nodules extended far into the periglandular fat (Figs. 3 & 9) and simulated a neoplastic process. The extracapsular cortical tissue in this case (Fig. 9) had to be differentiated from the "glandular" adipose tissue (Fig. 10) to which the periadrenal and other fatty deposits had been transformed in many other cases.[1] The residual cells and those in the proliferated nodules had a bizarre appearance. They were sometimes huge, with a cytoplasm deeply pigmented with brown granules and large, hyperchromatic nuclei (Fig. 8). The cellular groupings did not conform to the regular columnar and zonal arrangement of normal adrenal cortex (Fig. 12).

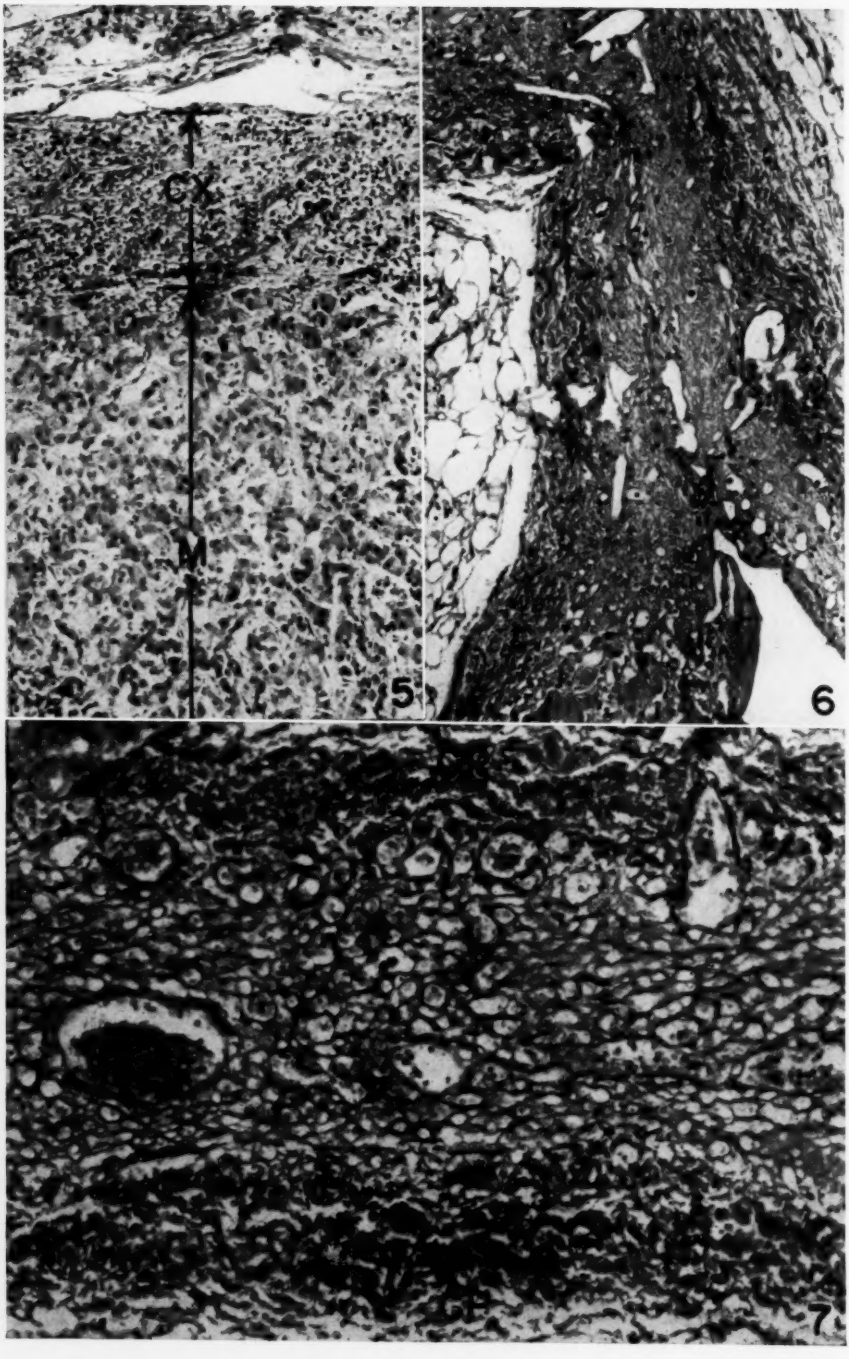
[1] *Note:* After this study was completed another example of extracapsular proliferation of cortical cells was called to my attention (Zamcheck). In that case the patient died of diphtheria but had adrenocortical contraction and other evidence of latent Addison's disease.

FIG. 1. Photomicrograph of a normal adrenal gland, taken at the same magnification as Figs. 2, 3 and 4, ×10.

FIG. 2. Contracted adrenal; the medulla makes up almost all of the parenchyma. Compare with the size of the normal adrenal in Fig. 1. The cortex has been reduced to a thin shell barely thicker than the capsule. Case 12. ×10.

FIG. 3. Nodular hyperplasia of cortical tissue obscuring the underlying loss of parenchyma in an adrenal gland which has undergone marked contraction. Proliferation of auxiliary cortical tissue in the periglandular fat has also taken place. Case 10. ×10.

FIG. 4. Atrophy of the adrenal cortex secondary to a hypothalamic-hypophyseal lesion. The uniform shrinkage without distortion or contraction differs completely from the destructive "atrophy" of adrenocortical contraction. ×10.


CX
M
5
6
7

Stains which brought out the argyrophilic reticulum clearly showed the unoriented and confused architectural patterns (Fig. 11).

Although there was no loss of medullary tissue, the medulla was often heavily infiltrated with small dark round cells. In 1 adrenal such cells formed clear-cut lymph follicles (Fig. 13).

MORPHOGENESIS

Most workers do not share Brenner's (1928) view that hyperplasia precedes rather than follows the cortical damage. The reasonable concept that necrosis of cortical cells precedes contraction is widely held. Duff and Bernstein (1933) and Susman (1930) pointed out that localized adrenal lesions resembling those diffusely distributed in adrenocortical contraction are seen from time to time at autopsy, and they suggested that summation of such damage could result in the full blown picture of adrenocortical contraction. It is true that focal necrosis and other degenerative lesions of the adrenal cortex are commonly encountered in a variety of conditions (Zamcheck), but such lesions probably could not result in the extensive, catastrophic, parenchymal destruction which is assumed to precede the ultimate contraction. Wells, Humphreys and Work (1937) pointed out that only massive necrosis could account for the final anatomic picture and expressed the opinion that an accumulation of minor atrophic lesions (Susman, 1936) could scarcely be adequate to produce it. The massive hemorrhage and necrosis of the adrenal which characterize the Waterhouse-Friderichsen syndrome could conceivably culminate in contraction, but there is no evidence to relate the two conditions. Signs of old hemorrhage were not seen in any cases of the Institute series. A history of episodes of weakness associated with infections suggested to Kiefer (1927) that toxic infectious processes might lead to adrenal contraction. In case 1 of the present series there was profound weakness following pneumonia.

Massive cortical degeneration, necrosis and inflammation, which might represent the hypothetical first stage in the development of adrenocortical contraction, were seen in 4 cases studied at the Institute. The clinical histories in these 4 cases are similar to those in the other cases of the series (Table 2). The adrenal changes seen microscopically in these 4 cases were as follows.

Case 22: Although no completely normal cortex remained, the

FIG. 5. The medulla (M) is preserved, but the cortex (CX) is reduced to a thin layer. Case 11. × 125.

FIG. 6. Extreme contraction of adrenal cortex. This section is taken from the peripheral portion, where medullary tissue is normally absent. A small amount of condensed interstitial tissue is all that lies between the apposed capsules. Case 8. ×125.

FIG. 7. Collapsed portion of adrenal cortex, similar to that illustrated in Fig. 6; stained for argyrophil reticulum (Wilder). Between the opposite capsules (CP) only a condensed network of the pre-existing reticular and sinusoidal structure remains. Case 12. ×240.

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medulla was intact. In most places the reticular fibers had disappeared or were damaged; focally, the original pattern was preserved. Most of the cortex consisted of blood-filled sinusoids arranged in the usual pattern, running perpendicular to the capsule. Between the sinuses were a few necrotic cortical elements, cell "ghosts," extravasated red cells and lymphocytes (Fig. 14). In a few places, almost invariably in the outer portion of the cortex, viable parenchymal elements were seen. Although some were arranged in a way which suggested the previous columnar pattern, others formed little nodular aggregates. Occasional collections of lymphocytes studded the degenerated cortex, and there was diffuse lymphocytic infiltration about the persisting cell clusters.

Case 23: The cortex showed a band-like zone of degenerated adrenal tissue which traversed the entire gland. The medulla was unaffected. The zona glomerulosa was for the most part spared, but the zona fasciculata showed degeneration or disappearance of the cellular elements, complete in some places and partial in others. In most places cell "ghosts," in the form of acidophilic vacuolated coagulated masses, were evident. The nuclei when present were pyknotic or crenated. The appearance was not unlike that of zonal necrosis of the liver (Fig. 15). The reticular framework was practically unaltered. Few reactive leukocytes were noted.

Case 24: The entire cortex was made up of columns of degenerating and hyperplastic parenchymal cells. Vacuolization of individual cells or tubular and glandular transformation of the entire cord (Fig. 16) characterized most of the tissue. Some cells had acidophilic, hyaline masses or inclusions in the cytoplasm. Precipitated protein formed lacy networks in the pseudoglandular cavities. Between the abnormally widely spaced cortical columns the condensed stroma was prominent, and the tissue spaces contained edema fluid, red cells and precipitated protein. Where "regenerative" features predominated, the "irritated"-appearing cells had ample granular cytoplasm and active looking "owl's eye" nuclei containing large nucleoli. The cells were grouped in cords several layers thick, and in some areas solid sheets were formed. Nodularity was not noted. The reticular fibers were destroyed in many places, but where they persisted the altered architecture of the cortical columns disturbed their patterns.

Case 25: The fascicular zone of the entire cortex exhibited disinte-

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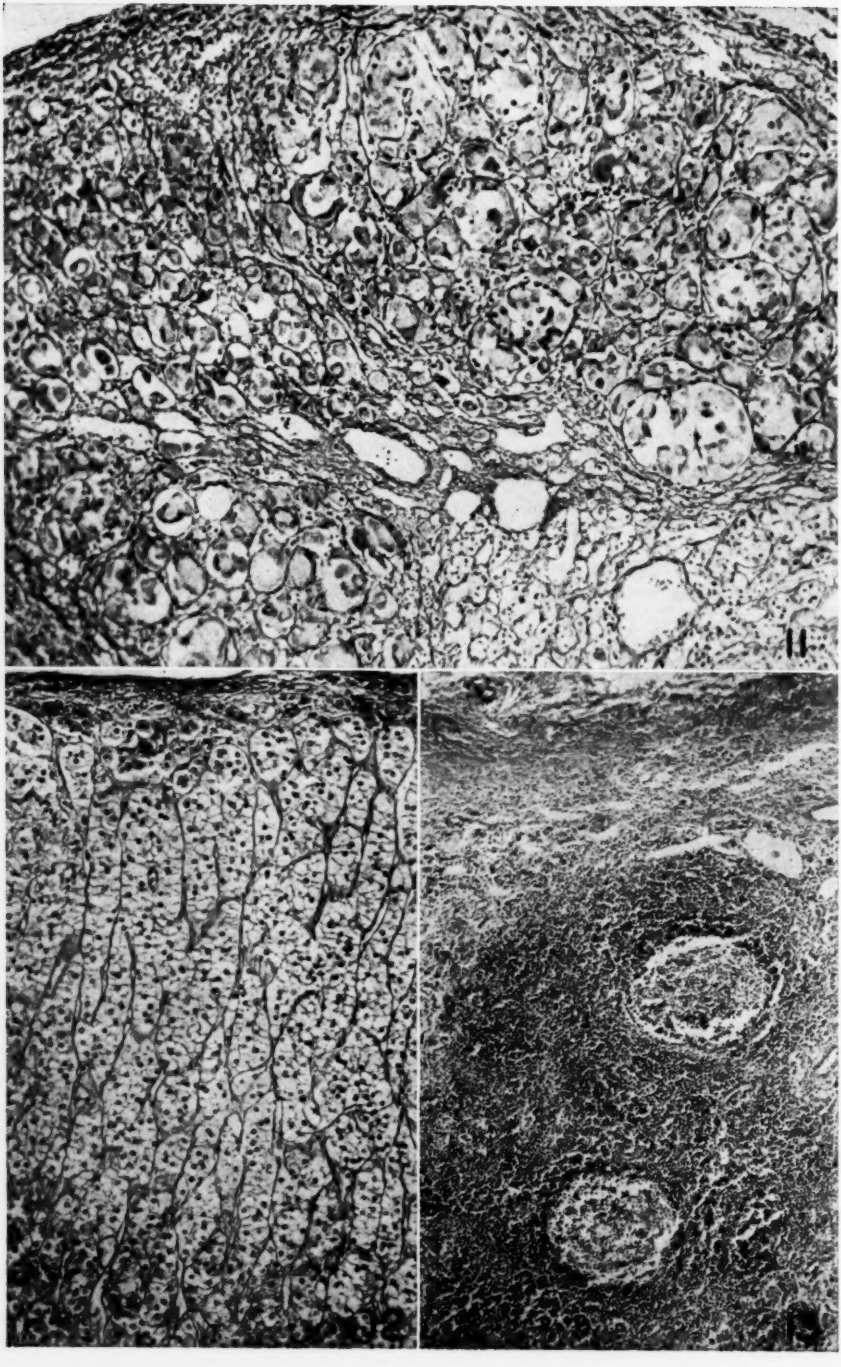
FIG. 8. Giant and distorted cortical elements are often present in contracted adrenal glands. Many such cells contain dark brown pigment. Case 11. ×200.

FIG. 9. In this instance of adrenocortical contraction there was marked secondary nodular hyperplasia; accessory cortical tissue extended far into the periadrenal fat. Extracapsular cortical cells are sometimes difficult to differentiate from fat cells which have undergone glandular or embryonal transformation (See Fig. 10). Case 10. ×125.

FIG. 10. Glandular fat in the adipose tissue surrounding a contracted adrenal. Such altered fat cells may be difficult to differentiate from adrenocortical elements. Case 11. ×210.



gration and lysis of the cortical cells (Fig. 17). Nuclear debris and granular cytoplasmic remnants were evident within the normally demarcated cortical columns. In many places leukocytes had infiltrated the destroyed cell cords. The reticulum was not significantly disturbed.

The lesions in cases 22, 23 and 25 consisted of massive necrosis and lysis of cortical cells. In case 22 collapse of the cortex had taken place. In case 24 "regeneration" or a comparable alterative process masked the striking degenerative aspects of the lesion. The picture in case 25 was more like that of acute "adrenalitis" than that in any other case. The fact that there was also leukocytic infiltration of lymph nodes in this case suggested that some septic process may have been responsible for the adrenal damage. In all 4 instances the processes duplicated what is seen in the necrotizing and reactive stages of acute destruction of hepatic parenchyma. It does not seem unreasonable to assume that such a state of affairs must precede the end stage of adrenocortical contraction.

ETIOLOGY

Congenital malformation, tuberculosis, syphilis, reaction to infection, vascular occlusion, intoxication with germanin, endocrine disturbances and virus infection have all been indicted as possible causes, but the etiology of adrenocortical contraction is still unknown.

Congenital absence or hypoplasia of the adrenal may be responsible for Addison's disease, but the difficulty in locating adrenals which have undergone extreme cortical collapse makes one wonder whether in many of the cases cited by Crosby (1930) "absence" of the gland may not have resulted from contraction rather than from faulty development.

There is only 1 case of Addison's disease in the files of the Institute in which the picture appeared to result from syphilitic destruction of the adrenal. Although spirochetes were not demonstrated in the gland, there was massive sclerosing obliteration of the parenchyma, and the patient had died of adrenal insufficiency while under treatment for taboparesis. The diffuse enlargement of the gland, the productive fibrosis and the sclerosing parenchymal destruction (Fig. 18) were entirely different from the features of contracted adrenals.

Rogoff (1936) mentioned finding acid-fast bacilli in an adrenal gland exhibiting "cytotoxic" atrophy, and Barker (1929) cited a case

FIG. 11. Cortical tissue in a contracted gland, illustrating disorganization of architecture and absence of normal cortical columns (See Fig. 12); stained for argyrophil reticulum (Wilder). Case 9. ×125.

FIG. 12. Normal adrenal cortex stained for argyrophil reticulum (Wilder), showing the typical columnar arrangement of parenchyma and the intercolumnar network of reticulum and sinusoids. Compare with the disorganized pattern in adrenocortical contraction (See Fig. 11). ×125.

FIG. 13. Heavy infiltration of the medulla by round cells in adrenocortical contraction. Their lymphoid nature is indicated by the formation of follicles. Case 2. ×75.

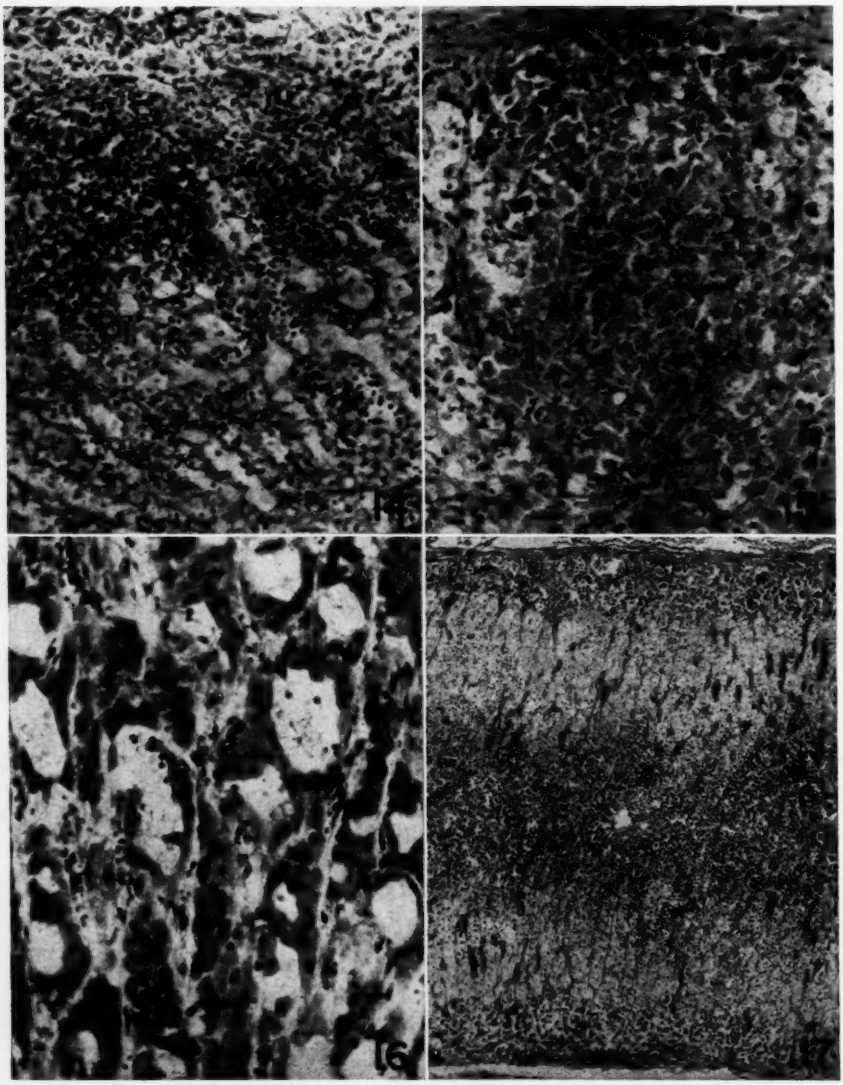


FIG. 14. Acute postnecrotic "collapse" of adrenal parenchyma. A few cortical cells remain immediately beneath the capsule. Most of the cortical elements have disappeared, leaving dilated sinusoids, reticular fibers and groups of round cells. Case 22. ×235.

FIG. 15. Acute zonal cortical necrosis of adrenal. A thin rim of relatively unaltered cortical cells remains immediately beneath the capsule. Most of the parenchymal elements have undergone coagulation necrosis and/or lysis. There are a few leukocytes. Case 23. ×160.

FIG. 16. Acute universal "glandular" degeneration of adrenal cortex. Cavities have

in which there was tuberculosis of one adrenal and atrophy of the other. However no one has suggested that the morphologic features of adrenocortical contraction resemble those of a tuberculous lesion.

It has recently been suggested (Rogoff, 1944), because ischemic damage to the adrenal which resulted in functional insufficiency has been produced experimentally in animals, that vascular occlusion could be held responsible for adrenal "atrophy" in Addison's disease. It is reasonable to assume, as did Rogoff (1936), that the Addison's disease which developed in a patient who had been subjected to an operation in the vicinity of the adrenal probably resulted from compromising of the vascular supply of the gland. There is no doubt that vascular occlusion can cause significant lesions in the adrenal and may even result in Addison's disease. For example, the Institute's files contain material from a patient in whom adrenal insufficiency developed after thrombosis of the adrenal vessels. The lesions were typical infarcts (Fig. 19). Another patient, who did not have Addison's disease, died 3 weeks after nephrectomy, with lesions in the adrenal. The segmental involvement, cicatricial fibrosis, obliteration of vessels and frank infarction which were present (Figs. 21, 22 & 23) bear no resemblance to the features of the usual instance of adrenocortical contraction but are strikingly similar to those of the lesions produced experimentally by vascular occlusion (Rogoff, 1944). Rogoff's view that a chronic inflammatory process might cause "cytotoxic atrophy" through vascular occlusion cannot be accepted because of the striking differences between ischemic lesions of the adrenal and those of adrenocortical contraction.

Wells, Humphreys and Work (1937) and, later, Tomlinson and Cameron (1938) reported cases in which it appeared that germanin (Bayer 205) might have been responsible for adrenal necrosis and contraction and Addison's disease. Since the drug was administered as a therapeutic agent to patients with pemphigus, the possibility must be considered that the adrenal lesions were associated with pemphigus rather than with germanin. However, the lesions of pemphigus described by Goldzieher (1945; 1946) and Guimaraes and Vieira (1945) were not those of adrenocortical contraction. Similarly, study of the adrenals in the Institute's collection of 20 cases of various types of pemphigus revealed only the nonspecific regressive and degenerative changes to be expected in a severe illness or in association with a widespread vesiculating dermatosis. Nothing approaching the picture of contraction was seen. Humphreys and Donaldson (1941) were able to produce adrenal damage by administration of germanin

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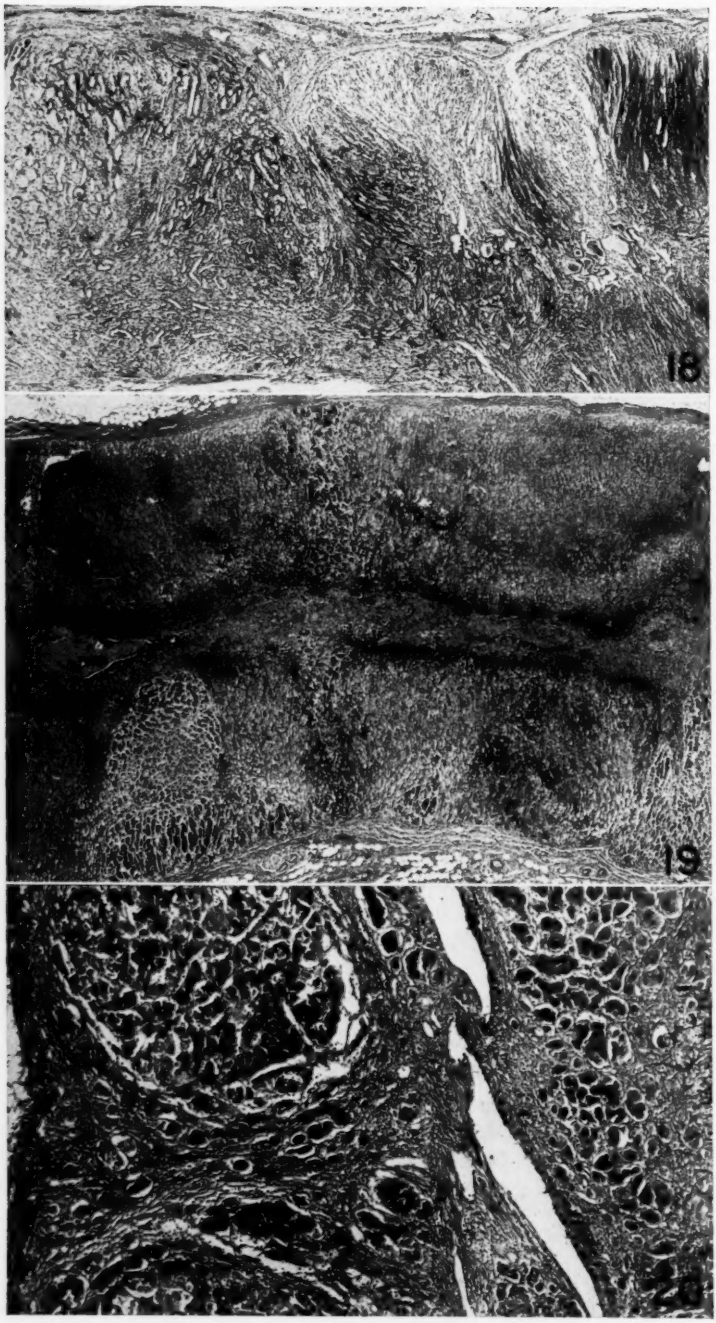
formed in the centers of many of the cords and the remaining cells are hyperplastic. Case 24. ×160.

FIG. 17. Acute cortical necrosis and "adrenalitis." The inner two thirds of the cortex is necrotic and infiltrated with leukocytes. Dilated sinusoids are prominent. Case 25. ×55.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to guinea pigs, but Wells, Humphreys and Work (1937) stated that it is improbable that a drug used as rarely as germanin could have been responsible for many instances of adrenocortical contraction. They suggested that clinicians should be on the alert for adrenal effects from other therapeutic chemicals in more common use than germanin. They also pointed out that although germanin was extensively used in the treatment of trypanosomiasis (African sleeping sickness), nothing in the literature (MacLean and Fairbairn 1932; MacLean, 1928) indicated that with such therapy trypanosomiasis was complicated by adrenal lesions. However, the clinical picture of adrenal insufficiency might be obscured in a Negro patient dying of sleeping sickness (Wells, Humphreys and Work 1937), and local conditions might interfere with the performance of complete autopsies.

One patient in this series had a previous operation for thyrotoxicosis, but no evidence has been brought forth that endocrine factors bear any etiologic relation to adrenocortical contraction. The atrophic adrenal glands seen in association with damage to pituitaries or hypothalamic lesions (Fig. 4) might at times be confused with contracted adrenals, but the pattern of uniform atrophy without distortion or parenchymal loss is usually easily distinguished from that of adrenocortical contraction (Crooke and Russell 1935). I have, however, seen an adrenal lesion, probably endocrine in origin, which was almost indistinguishable from that of adrenocortical contraction; the patient, a man of 53, had been castrated and treated with estrogens (a-estradiol diproprionate and diethylstilbestrol), for carcinoma of the prostrate. Clinical evidence of adrenal insufficiency was not noted. Although the adrenals were not small there were irregular areas of cortical collapse and nodular proliferation (Fig. 20).

The observation of intranuclear inclusions in a contracted adrenal from a patient with Addison's disease suggested to Weiner (1936) "another interpretation." There is no evidence to support his implication that an infectious process might be responsible for the adrenal lesions, although the lesions of the liver in epidemic hepatitis (Lucké, 1944), which is known to be caused by a virus, are comparable to the adrenal changes in adrenocortical contraction.

COMMENT

There is no doubt that many features of adrenocortical contraction duplicate those of the lesions of certain diseases of the liver. Dis-

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FIG. 18. Syphilis of the adrenal in a patient who died of Addison's disease. The enlarged gland shows proliferation of both reticulum and collagen, with sclerotic destruction of the parenchyma. The pattern is entirely different from that of adrenocortical contraction (Wilder stain). × 15.

FIG. 19. Infarction of the adrenal. Although some cortical islands remain the patient had adrenal insufficiency. ×15.

FIG. 20. Adrenal lesions in a castrated man who had been treated with estrogens for carcinoma of the prostate. There are shrinkage of parenchyma and prolliferation of atypical cortical elements (Wilder stain). ×70.

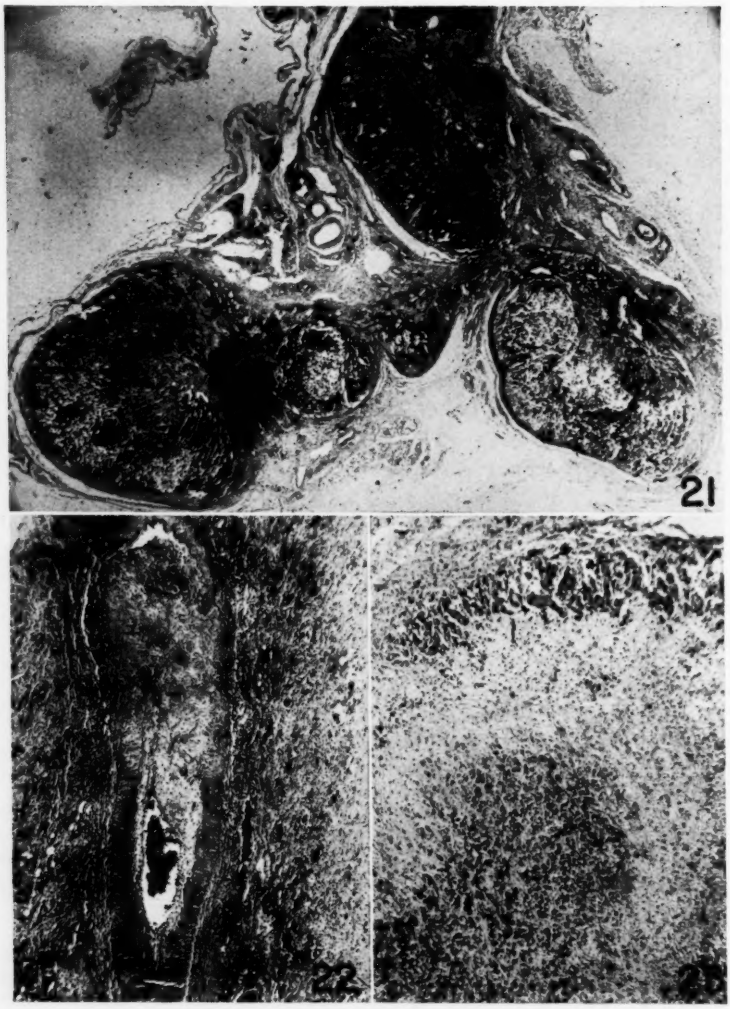

FIG. 21. Cicatrization of the adrenal resulting from vascular damage incurred at the time of nephrectomy, 3 weeks before death. Such segmental fibrosis does not occur in adrenocortical contraction. A damaged vessel and a frank infarct in this adrenal are shown in figures 22 and 23. ×10.

FIG. 22. Almost complete obliteration of an adrenal vessel 3 weeks after nephrectomy. This vascular lesion was present in one section of the adrenal gland illustrated in Fig. 21. Vascular lesions are not seen in adrenocortical contraction. ×30.

FIG. 23. Infarct of adrenal, 3 weeks after nephrectomy. This lesion was present in one section of the adrenal gland illustrated in Fig. 21. Infarcts are not encountered in adrenocortical contraction. ×60.

appearance of parenchymal cells, condensation of reticular stroma, absence of cicatricial or replacement fibrosis and secondary nodular proliferation of abnormal tissue are all encountered in so-called acute yellow atrophy, toxic "cirrhosis" and some forms of hepatitis. This similarity is not surprising in view of the fact that both adrenal and liver are composed of columns of parenchymal cells delimited by networks of reticulum and sinusoids. The analogies between hepatic and adrenal reactions are emphasized even further when one recalls that "vascular" disease of the liver may superficially simulate hepatic collapse despite the fundamental difference in pathogenesis. Hepatic parenchymal necrosis, atrophy and collapse and "toxic cirrhosis" can usually be distinguished from syphilitic hepar lobatum and the similar hepatic picture occasionally produced by periarteritis nodosa, and the ischemic adrenal lesions should similarly be distinguished from adrenocortical contraction.

The designations of cirrhosis and atrophy as applied to hepatic necrosis and collapse have been objected to as not being accurately descriptive of the fundamental process. Similarly, no uniformly acceptable term has been devised for the comparable adrenal lesion. A variety of names have been applied to the condition, such as primary atrophy, idiopathic atrophy, cytotoxic atrophy, cytotoxic necrosis, contraction, cirrhosis, primary contracted adrenal gland, inflammatory granular atrophy and adrenalitis. Since none of these appellations is accurate and no suitable term is at hand, the nonspecific descriptive designation adrenocortical contraction has been employed despite the obvious objections.

Many workers (Langerhans, 1903; Steinbisz, 1926; Wells, 1930) have concluded that the bizarre nodules of cortical tissue which lack the normal architectural pattern (Kiefer, 1927) and contain abnormal cellular elements represent hyperplastic or regenerative reactions compensatory and secondary to the primary degeneration or necrosis. Such "regenerative" nodules, however, are not peculiar to adrenocortical contraction; similar nodules are seen (Barker, 1929; Guttman, 1930) in the residual tissue of adrenals destroyed by tuberculosis. Compensatory hyperplasia has even been noted in ectopic adrenal tissue in the epididymis and the broad ligament of 2 patients suffering from Addison's disease (Nicholson, 1922).

The finding of abundant "hyperplastic" adrenocortical tissue in patients who had clinical evidence of adrenal insufficiency, noted in several cases in the Institute series and previously reported in the literature, at first glance implies a contradiction between the morphologic and functional aspects of the disease process. This apparent disparity recalls instances in which patients died of hepatic insufficiency despite the presence of enormous masses of viable hepatic tissue, in the form of hyperplastic nodules. It has been suggested that such hepatic nodules are functionally useless because they do not have

the proper vascular connections and are not arranged in the normal lobular pattern. As in the thyroid, where hyperplasia may be associated with hypofunction, hyperplastic hypertrophic cells may be overworking, overstimulated cells which are not delivering a normal secretory end product to the blood stream.

The "glandular" or "embryonal" reversion of the adipose tissue, which was noted in the periadrenal tissues and in the fat in other locations, was striking. Such transformation of fat is not a specific process; it is encountered in a variety of emaciating diseases and is common in children. However, such marked alteration in a condition in which there is a deficient production of steroid hormones is of interest, since it is known that fat soluble hormones are at least stored in fat. Embryonal transformation of fat probably accounts for the pigmentation of adipose tissue described by Duffin (1943). Altered periadrenal fat must be differentiated from extracapsular cortical tissue in some instances.

The pericarditis present in 3 cases appears to be an unusual complication of Addison's disease. Terminal infection appears unlikely but not impossible as a cause, and an azotemic exudate cannot be altogether ruled out. In 2 cases the pericarditis had led to the diagnosis of coronary occlusion.

The danger of temporizing with the medical emergency of adrenal insufficiency is brought out by the clinical histories. Addison's disease should be seriously considered in the differential diagnosis of acute and subacute disturbances referable to the gastrointestinal tract, psychotic reactions and sudden collapse.

SUMMARY

Between December 1941 and December 1946 pathologic material from 15 patients who exhibited adrenocortical contraction at autopsy was received at the Army Institute of Pathology. During the same period specimens from only 10 patients with Addison's disease caused by tuberculosis were accessioned. Prior to World War II, only 6 cases of adrenocortical contraction had been studied at the Institute, as contrasted with 21 cases of tuberculous Addison's disease.

The clinical syndrome was in many instances so atypical and confusing that the diagnosis of adrenal insufficiency was not entertained, particularly when the disease ran a short, fulminating course. Acute gastro-enteritis, poisoning, ruptured peptic ulcer, coronary occlusion, psychosis, intracranial hemorrhage and myasthenia gravis were all simulated. The danger of temporizing with the medical emergency of adrenal insufficiency was underlined by the repeated occurrence of sudden collapse and death.

The morphologic picture in the 21 cases of full blown adrenocortical contraction ranged from that of pure destructive atrophy, or

collapse of the cortex to that in which the regeneration of cortical cells and nodules in atypical patterns overshadowed and masked the underlying atrophy. Although the early stage of "atrophy" is rarely encountered in material obtained at autopsy, the Institute files contain 4 examples of cortical degeneration, necrosis and inflammation so pronounced that one could conceive of their being the hypothetical precursors of adrenocortical contraction.

The lesions in adrenocortical contraction differ strikingly from those caused by vascular occlusion, syphilis and tuberculosis. They bear a strong resemblance to the lesions of necrotizing hepatic injury and its sequelae.

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PROGESTERONE AND DESOXYCORTICOSTERONE IN THE STEROID CONTROL OF THE GONADOTROPHIC FUNCTION OF THE HYPOPHYSIS

(Exemplified by the Behavior of the Intrasplenic Ovarian Graft in the Guinea Pig)

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LARGE blood follicles and cystic follicles appear in intrasplenic ovarian grafts in the castrated guinea pig (Lipschutz, 1946).[1] When the second ovary is left *in situ*, these blood follicles or cystic follicles do not occur. This gives full evidence that the phenomenon is due to the hypophysis being freed from the control by the ovary. Besides formation of blood follicles luteinization also becomes abnormal. There is partial luteinization of the wall of cystic hemorrhagic or non-hemorrhagic follicles, true lutein cysts can be found. Clusters of luteinized cells are scattered in the stroma. But corpora lutea are small at two months after transplantation; most of the corpora lutea do not attain the diameter of normal corpora lutea. From the third month on luteinization becomes overwhelming; large corpora lutea become numerous. At about ten months a luteomatous condition is attained though blood follicles continue to be found (Lipschutz *et al.*, 1946). The luteomatous degeneration of the ovary has been described by Biskind and Biskind (1944) in the rat and by various authorities in the mouse (Li and Gardner, 1947; Furth and Sobel, 1947). The luteomatous neoplastic condition in rats and mice, in any case in certain strains, is attained sooner than in the guinea pig; blood follicles have not been described by the authorities working with intrasplenic grafts in rats and mice. When comparing the ovarian condition in similar experiments in mice and guinea pigs it becomes evident that the evolution of the graft, including its neoplastic condition due to the rupture of the normal ovarian-hypophyseal relationship, differs with the species.[2] In the guinea pig the evolution of the luteomatous condition

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[1] Described in 1944 in the Thesis of M.D. of H. Ponce de León and subsequently in those of E. Woywood and O. Gay.

[2] The senior writer was kindly given the opportunity to examine the slides of Dr. W. U. Gardner in Yale (intrasplenic ovarian grafts in mice).

is so slow that the intrasplenic graft offers a very good opportunity for reexamining, by administration of hormones, the steroid control of the gonadotrophic function of the hypophysis *in situ*, freed previously from gonadal control.[3] Certain difficulties may arise from adhesions between the spleen and the abdominal wall which allow a escape of an unaccountable quantity of estrogen into the general circulation. But in work with guinea pigs one is not much hampered by this accident when operating under aseptic conditions.

In the present paper we shall deal with experiments in which the action and interplay of α-estradiol, progesterone and desoxycorticosterone have been studied. Experiments with testosterone and other 3-keto-steroids are in progress.

PROCEDURE

Both ovaries were removed and one of the ovaries was grafted into the spleen. Two weeks later a pellet of the respective steroid, or steroids, was implanted subcutaneously. The animals were sacrificed two months after ovarian transplantation. The part of the spleen containing the ovary was fixed in Bouin's fluid to be cut two days later with the razor into slices about 1 mm. thick; presence of blood follicles, tertiary follicles and corpora lutea was noted. Representative slices were chosen in each case for microscopical examination. The corpora lutea were counted in a number of sections and the maximal number encountered in *one* section was taken as the "number of corpora lutea." Two diameters of the corpora lutea were measured in sections in the usual way; the "maximal corpus luteum diameter" (the average of the two maximal diameters measured) was thus determined for each animal. Similar measurements were made also for blood follicles and tertiary follicles. As to the quantitative statements about corpora lutea there were sometimes unsurmountable difficulties due to the irregularities of luteinization referred to above. Such cases were omitted.

EXPERIMENTS WITH α-ESTRADIOL

Results are summarized in Table 1.

When no steroid is added most grafts show blood follicles. Their incidence may vary from one series to another but their frequency is always overwhelming. In the present series *Ib* two thirds had blood follicles. Their diameter was in most cases greater than 1 mm., i.e. to the maximal diameter of a tertiary follicle we have found in a series of six normal adult guinea pigs; the diameter of blood follicles may attain as much as 2 mm. or more. The diameter of non-hemorrhagic tertiary follicles varied greatly; they were often cystic, attaining a diameter double that of the normal. With α-estradiol (group II) abnormal follicular growth was definitely counteracted, there being no blood-nor cystic-follicles. None of the steroids administered simultaneously with α-estradiol—progesterone (V), desoxycorticos-

[3] This paper was already written when the work of Jungk, Heller and Nelson (1947) and of Heller and Jungk (1947) on the rat came to our knowledge.

terone acetate (VII)—were able to counteract this controlling action of the estrogen.

Another highly interesting aspect in our work was the considerable increase in luteinization under the influence of estrogen. Though, as already explained, corpora lutea are present and sometimes even numerous in the intrasplenic graft of the castrated guinea pig at two months after transplantation, they only exceptionally attain at this time the diameter of a normal corpus luteum, which is about 1.5 mm. On the contrary, when estrogen is administered, corpora lutea can be

TABLE 1

Group	Steroid	Absorb. per day μg.	Number of animals				Percentage of animals		
			Total (n)	With blood foll.	With C.l. (n_1)	With large C.l.*	With blood foll.	With C.l.	With large C.l.**
Ia*	None	0	19	18	12	2	95	63	10
Ib	None	0	29	19	12	2	66	41	7
II	α-estrad.	28–65	12	0	12	10	*0*	*100*	*80*
III	progest.	91–160	17	10	8	3	59	65	18
IV	progest.	180–469	23	14	4	1	61	17	4
V	α-estrad. and progest.	34–114 / 269–400	14	0	1	0	*0*	*7*	*0*
VI	desoxycort. ac.	178–320	8	4	1	0	50	13	0
VII	estrad. and desoxycort. ac.	17–72 / 180–490	13	0	2	0	*0*	*15*	*0*

* Ia is from a former series (Lipschutz *et al.*, 1946); for comparison with Ib.
** Diameter of 1.4 mm.

found in all animals; at two months 80 per cent of the animals, as against about 10 per cent without estrogen, have corpora lutea of at least a normal if not a larger diameter.

The degree of luteinization may be conveniently expressed in an index multiplying the average number of corpora lutea by the cube of the average of the maximal diameters in animals of the group. The procedure is indeed an arbitrary one, and as shown in the last column of Table 2, the index may vary greatly in animals without steroids. But the increase of corpora lutea formation under the influence of estrogen is overwhelming, there being a great increase both in the number and in the diameter of corpora lutea.

These findings with α-estradiol are corroborative of what is known from former manifold studies in which very different methods have been used.

RESULTS WITH PROGESTERONE

It may be said beforehand that these results, as shall be shown in the last section of this paper, acquire especial interest in relation with the findings and concepts of Smith and Smith (1946) on the role

of progesterone in the dynamics of the ovarian-hypophyseal relationship.

The incidence of blood follicles and corpora lutea was only influenced insignificantly, if at all, by the administration of progesterone alone (group III, table 1). With large quantities of the steroid diminution of the incidence of corpora lutea (compare groups IV with I) is rendered probable by our results. But interference of progesterone became fully evident in those experiments in which progesterone was administered simultaneously with α-estradiol (compare group II and V).

Progesterone even when given in large quantities (many times

TABLE 2

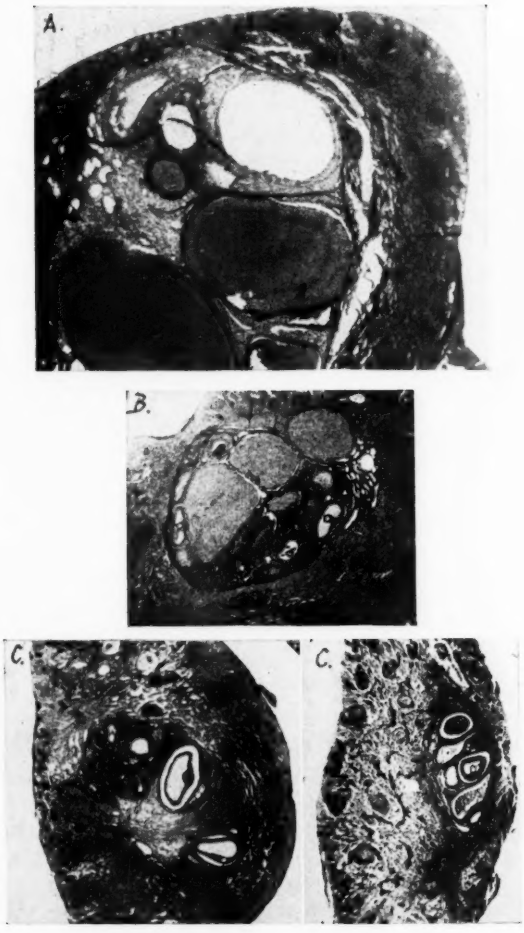
Group	Steroid	Corpora lutea			Index of luteinization
		Total	Average per animal* (a)	Largest diameter** (b) mm.	axb^3+10
Ia	None	28	1.45	0.96	13
Ib	None	21	0.72	1.06	9
II	α-estradiol	26	2.17	1.7	106
III	progest. small dos.	17	1.00	1.08	13
IV	progest. large dos.	6	0.26	1.16	4
V	α-estr. and progest.	1	0.07	0.4	<1
VI	desoxyc. ac.	1	0.13	0.76	<1
VII	α-estrad. and desoxyc. ac.	3	0.23	1.0	2

* Total number of corpora lutea divided by total number of animal (*n* in table 1).
** The sum of the largest diameters divided by the number of animals with corpora lutea (n_l in table 2).

those of α-estradiol) does not counteract the controlling action of α-estradiol as to blood follicles. There were no blood follicles in group V. Neither were there cystic tertiary follicles. On the contrary, the luteinizing influence of the estrogen is definitely inhibited by progesterone (table 1). All animals had corpora lutea when α-estradiol was given; addition of progesterone caused a drop from 100 per cent to 7 per cent. Even more striking was the drop of incidence of *large* corpora lutea—from 80 to 0 per cent. The index of luteinization (table 2) dropped from 106 to less than 1.

EXPERIMENTS WITH DESOXYCORTICOSTERONE ACETATE.

This steroid was tried alone (group VI) or in combination with α-estradiol (group VII). Desoxycorticosterone acts similarly to progesterone. There was no significant influence on the incidence of blood follicles. Inhibition of corpora lutea formation in group VI was no less pronounced than with similar quantities of progesterone (group IV and VI). There was also a definite inhibition of the luteinizing



EXPLANATION OF FIGURES

Intrasplenic autoplastic grafts in castrated guinea pigs. 60 to 65 days after transplantation, and 39 to 49 days of action of different steroids.—A. *Progesterone.* Large hemorrhagic follicles as in animals without steroids. Cystic follicle. Distension of the spleen (CI.85).—B. *α-estradiol.* No hemorrhagic follicles. Corpora lutea always present. Tertiary follicles small. No distension of spleen (CI.26).—C. *α-estradiol and progesterone.* No hemorrhagic follicles. No corpora lutea. Tertiary follicles small. No distension of spleen (CI.94 and 97).—All figures augment. ×10.

faculty of α-estradiol without the controlling action of the latter as to follicular growth being interfered with (group II and VII).

DISCUSSION

The results obtained with reference to the interference of progesterone in ovarian events can be summarized in the following two

statements: (1) interference of progesterone is as fundamental for the maintenance of the ovarian cycle as is α-estradiol; (2) under certain quantitative conditions progesterone counteracts or antagonizes the luteinizing influence of α-estradiol. As to luteinization there is definite evidence not only of a differential but also of an antagonistic behavior of the two steroids: α-estradiol calls for luteinization, and progesterone holds luteinization in abeyance. This differential and antagonistic behavior of the two steroids is an experimentally established fact which is highly suggestive of the concept that the normal sequel of the follicular and luteic phases of the ovary are under the dependence of an interplay of the two steroids mentioned. It is now that the question arises about how and where this interplay takes place.

Three possibilities have to be taken into consideration: (a) an interplay of the two steroids *in the ovary itself;* (b) an interplay in certain phases of the *metabolism and excretion of the steroids;* (c) an *intrahypophyseal interplay* of the two steroids controling the timing as to the release of the follicle stimulating and luteinizing gonadotrophic hormones.

It cannot be denied that estrogen may stimulate follicular development by direct action on the ovary (Pencharz, 1940; Williams, 1940, 1944; Simpson *et al.*, 1941). But the changes induced by the direct action of estrogen on the ovary of the hypophysectomized rat are just the opposite of those we are dealing with here: follicular development in the hypophysectomized rat is enhanced by estrogen, whereas it is "controlled" or "limited" in the intrasplenic graft in the guinea pig by estrogen administration. Direct action of estrogen on the ovary cannot serve as an explanation for those profound ovarian events which are consequent to the administration of estrogen in experiments with intrasplenic ovarian grafts. More significant, from the point of view of the dynamics of the steroid control of the follicular events in the intrasplenic graft, are the older findings of Westman and Jacobsohn (1937) and of Robson (1937) in the hypophysectomized pseudopregnant rabbit in which the corpus luteum remained histologically normal when estrogen was administered. No data are available about a direct action of progesterone on the ovary.

The statements of Smith and Smith (see summary 1946) relative to the enhancement of metabolic conversion of estradiol by progesterone are very suggestive. But even when fully admitting this type of progesterone interference in our experiments this would not mean dropping definitely the concept of progesterone partaking in some way in the steroid control of the hypophyseal gonadotrophic function by direct action on the anterior lobe.

The concept that estrogen enhances the release of the luteinizing hormone would be in accord with our present results. This is also in full accord with a former statement of the senior writer that whereas the faculty of the anterior lobe of the male donor rat to induce lutein-

ization in an immature female recipient diminishes considerably when estrogen has been previously administered for a certain time to the donor there is no concomitant diminution of the faculty to induce estrus in the immature recipient and to elicit an increase of the uterine weight (Lipschutz, 1935). It is very likely that the estrogen-induced release of the luteinizer has been counteracted by progesterone in our present experiments with intrasplenic grafts. But as evidenced by the condition of the graft, release of the luteinizer is not the only action of estrogen. There is also the fact that production of blood follicles is controlled by estrogen. This may be interpreted in the sense that estrogen puts a brake on an uncontrolled release of the follicle stimulating hormone. Should interference of progesterone in the conversion of estrogen be the only mechanism in play, without any action of progesterone on the anterior lobe, one should suppose that controlling the release of F.S.H. and enhancing the release of L.H. take place at different quantitative levels of estrogenic action. One should then assume that with larger quantities of progesterone administered simultaneously with the estrogen it should be possible not only to counteract luteinization as in fig. C but also to revert the ovary to a condition as in fig. A. The quantities of progesterone used in our experiments and still insufficient to counteract *both* aspects of estrogenic action were indeed considerable compared with those of α-estradiol administered simultaneously (269 to 400 μg of progesterone daily for 45 days; (table 1). Experiments with smaller quantities of the estrogen and larger quantities of progesterone are needed to settle this question.

On the other hand an action of progesterone on the anterior lobe with inhibition of the release of L.H. has been stated in the rat by Astwood and Fevold (1939) with quantities of 0.1 to 1 mg. of progesterone daily. On the contrary, inhibition of the release of F.S.H. was found only exceptionally, even with quantities so great as 3 to 6 mg. of progesterone daily during five to six weeks (Cutuly, 1941).

The slight action of progesterone and desoxycorticosterone, in absence of any estrogen (see group IV and VI, table 1 and 2), if significant, would be in favor of the concept that progesterone may act under certain quantitative conditions, also directly on the anterior lobe. But the objection may be raised that the diminution of luteinization in the two groups (IV and VI) compared with groups Ia and Ib, was due to conversion—in IV and VI—of those small quantities of estrogen which may have escaped inactivation. The results of Smith (1944, 1945) with Westerfield's lactone, an oxydative product of estrone, whose action on the hypophysis cannot be counteracted by progesterone, do not favor either the concept of an action of progesterone on the anterior lobe.

Whether progesterone interferes in the events in the intrasplenic graft exclusively by enhancing conversion of α-estradiol, or by direct intrahypophyseal action, it seems now clear enough that the problem

of the steroid control of the gonadotrophic function of the anterior lobe is very far from being exhausted by the oversimplified concept that estrogen serves as a "brake"of this function; even with quantities certainly much greater than physiological ones such a "brake"—in an absolute sense of the word—has not been established in our work. It may be definitely stated, however, that in the steroid control of the gonadotrophic function of the anterior lobe progesterone is certainly as fundamental as is estrogen. The work of Smith and Smith and so also our new findings make it easy to understand why there must be in the body intrahepatic inactivation not only of estrogen but also of progesterone (Dosne, 1944) though in a quantitatively limited or controlled manner (Bruzzone and Cuevas, unpublished).

Our finding that desoxycorticosterone controls the condition of the intrasplenic graft in the same direction as does progesterone also deserves attention as it constitutes another example of the overlapping of actions of progesterone and desoxycorticosterone. Quantitative data are as yet not available. But the statement is of importance that the quantities of desoxycroticosterone acetate used in our experiments were not greater than those necessary for the survival of the suprarrenalectomized guinea pig (Bruzzone *et. al.*, 1946) and they are still unable to produce any disturbance of the ionemia in normal guinea pigs (Alvarez and Fuenzalida, 1946).

SUMMARY

The intrasplenic ovarian graft in the castrated guinea pig has been made use of for the study of the seroid control of the gonadotrophic function of the anterior lobe, *in situ*, freed from the control of the endogenous ovarian steroids inactivated in their passage through the liver.

Blood follicles and cystic follicles which are a most characteristic feature of the intrasplenic ovarian graft in the castrated guinea pig fail to appear when α-estradiol is administered.

On the contrary, α-estradiol greatly enhances production of corpora lutea which, when no estrogen is administered, are scarce in similar grafts in the castrated guinea pig at two months after transplantation.

When progesterone is administered follicular development with production of blood follicles and cystic follicles is not inhibited; luteinization is slightly inhibited.

When α-estradiol and progesterone are administered simultaneously there are no blood follicles and there is no luteinization, that is progesterone counteracts the stimulative action of estrogen on luteinization but not the estrogenic control of follicular growth.

The problem of the interplay of the two essential ovarian steroids in the control of the hypophyseal gonadotrophic function is discussed.

Desoxycorticosterone acts similarly to progesterone.

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THE INFLUENCE OF HYPOPHYSECTOMY, THYROIDECTOMY, AND OF BOTH HYPOPHYSECTOMY AND THYROIDECTOMY UPON THE FAT CONTENT OF THE LIVER OF THE DOG[1]

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THE CONTROL exercised by pituitary and thyroid glands upon lipid metabolism has been the subject of earlier reports from this laboratory (Chaikoff *et al.*, 1936; Chaikoff *et al.*, 1941; Entenman *et al.*, 1942a and b). Hypophysectomy *per se* has but little effect on the levels of the blood lipids of the dog (Chaikoff *et al.*, 1936). Provided caloric intake is not restricted, thyroidectomy, on the other hand, produces changes in the concentration of blood lipids of this animal (Chaikoff *et al.*, 1941). Definite elevations in the levels of blood cholesterol esters were observed as early as 7 days after excision of the thyroid gland, and by the time 29 days had elapsed definite increases in total fatty acids were also found. Not all blood lipid constituents, however, responded to the same degree; phospholipids and free cholesterol seldom showed pronounced increases after thyroidectomy. It was later shown that the high levels of blood lipids produced by extirpation of the thyroid glands are dependent upon the nutritional state of the animal (Entenman *et al.*, 1942a). The rise in cholesterol esters and total fatty acids can be inhibited by fasting or by limitation of caloric intake.

In dogs deprived of both hypophysis and thyroid, the blood lipids may rise to extraordinarily high levels (Entenman *et al.*, 1942b). The abrupt and striking rise in blood lipids that occurs after removal of the second gland, as compared with the lack of response after hypophysectomy alone, suggested that thyroid deficiency is the primary stimulus to the blood-lipid rise of the doubly operated dogs. The more uniformly high blood-lipid levels in hypophysectomized-thyroidectomized dogs than in thyroidectomized dogs was ascribed to a depression of whatever accessory thyroid tissue may have remained after thyroidectomy.

It is shown in the present investigation that the control of these

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glands over lipid metabolism is not limited to blood. In dogs deprived of the thyroid alone, fatty livers *may* be present, but in those deprived of both glands fatty livers are an invariable occurrence. The lipid changes in the livers in these animals thus parallel those found in the blood.

EXPERIMENTAL

Hypophysectomized Dogs—Throughout the periods of observation each dog received twice daily, at 8:00 A.M. and at 4:00 P.M., 15 gm. of lean meat per kilo, 10 gm. of sucrose, 4 gm. of bone ash, and 1 gm. salt mixture.[2] Vitamin supplements were provided by the addition of 3 cc. Sardilene[2] and 5 cc. of Galen B.[2] After receiving this diet for 2–3 weeks the dogs were hypophysectomized by a method previously described (Dandy and Reichert, 1938). In the procedure used in this study no cautery was applied to the base of the brain. In most cases the appetites of the dogs were excellent after hypophysectomy.

Thyroidectomized Dogs—The procedure employed for excision of the thyroid glands has already been described (Chaikoff *et al.*, 1941). Before the operation dogs T2, T3, T5, T12, T13, and T14 were fed twice daily a diet of 15 gm. lean meat and 5 gm. of sucrose per kilo per day. In addition each dog received daily 2 gm. of the salt mixture, 5 gm. of bone ash, 3 cc. of Sardilene oil and 5 cc. of Galen B. The detailed dietary treatment of these dogs has also been described elsewhere (Entenman *et al.*, 1942a).

The dietary intake of dogs T6, T7, T16, T17, and T19 was kept constant throughout the experiment. Each dog received twice daily 15 gm. of lean meat per kilo, 10 gm. of sucrose and the vitamin and salt supplements described above.

Hypophysectomized-Thyroidectomized Dogs—For 2–3 weeks after admittance to the laboratory each dog (except HT15) was fed twice daily 15 gm. of lean meat per kilo, 1 gm. salt mixture and 2 gm. bone ash. HT3, HT6, HT8, HT10, and HT11 received 2.3 gm. sucrose per kilo, whereas HT18, HT21, and HT22 received 10 gm. sucrose per meal. HT15 was fed 150 gm. of whole ground fish (Balto dog food) and 50 gm. of sucrose per meal. Each dog also received 3 cc. of Sardilene oil and 5 cc. of Galen B in the morning meal. The dietary intake of all dogs except HT15 was constant throughout the experimental period.

After a suitable control period, each dog was subjected to complete excision of its pituitary gland. Four to 6 weeks later their thyroid glands were removed. In all cases one or two of the parathyroid glands were preserved with their blood supply intact. All other parathyroid tissues found were transplanted into neighboring muscle. For the first two weeks after the removal of the thyroid glands each dog was fed 5 gm. of calcium lactate and 100 cc. of whole milk per meal in addition to the other dietary constituents. In all dogs except HT11, appetite was lost after removal of the second gland. The exact caloric intake recorded for each dog was, however, maintained by forced feeding.

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[2] In all diets referred to here Cowgill's salt mixture was used (Cowgill, 1923). Each cc. of Sardilene contained not less than 100 A.O.A.C. chick units of vitamin D and 600 U.S.P. units of vitamin A. The vitamin content of Galen B has been previously recorded (Montgomery *et al.*, 1941).

At the end of the periods of observations listed in the tables the dogs were anesthetized with nembutal and the livers ground, sampled, and analyzed as described in a previous paper (Chaikoff and Kaplan, 1934). Tissues were also taken for histological examination.

Completeness of hypophysectomy was established in all dogs at necropsy.

RESULTS

Hypophysectomized (H) Dogs—It has been shown elsewhere that at early intervals after excision of the pituitary gland, namely 2.5–4

TABLE 1. EFFECT OF HYPOPHYSECTOMY ON PLASMA AND LIVER LIPIDS OF THE DOG

Dog no.	Body weight		Period after hypophysectomy	Plasma lipids						Liver	
				Cholesterol			Phospholipids	Total fatty acids	Total lipids	Weight	Total fatty acids
	Initial	Final		Total	Free	Ester					
	kg.	kg.	months	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	gms.	per cent
H25	7.2	9.7	24	114	52	62	275	475	589	220	2.2
H26	7.6	8.1	24	109	58	51	204	372	481	170	1.9
H27	7.2	7.8	24	73	29	44	192	284	357	152	4.9
H28	6.2	8.2	24	105	28	77	313	426	531	176	2.7
H29	6.4	9.1	24	134	34	100	246	518	652	196	3.9
H32	4.1	8.3	32	82	39	43	400	552	634	265	3.8
H33	5.5	6.7	32	81	37	44	318	499	580	212	3.2

TABLE 2. EFFECT OF THYROIDECTOMY ON LIVER LIPIDS OF THE DOG

Dog No.	Body weight			Period after thyroidectomy	Liver	
	Initial	At thyroidectomy	Final		Weight	Total fatty acids
	kg.	kg.	kg.	days	gms.	per cent
T6	13.3	13.3	11.7	197	446	2.3
T3	10.5	11.1	10.4	237	260	6.4
T16	8.7	8.7	8.0	365	253	6.6
T17	8.5	9.2	9.4	365	187	7.8
T19	9.0	9.0	9.2	365	252	11.5
T13	10.5	10.7	9.6	370	250	8.2
T7	8.5	8.6	8.5	387	201	5.9
T12	9.0	9.3	12.5	460	417	21.7
T14	12.5	13.0	15.3	460	419	20.2
T2	7.7	7.9	12.7	799	432	21.5
T5	9.5	9.9	13.2	799	431	22.7

months, the fatty-acid content of the liver remains within normal levels (Chaikoff *et al.*, 1936). The values recorded in Table 1 leave no doubt that the absence of this gland for as long as 32 months (960 days) is also without effect upon the lipid content of the liver.

Thyroidectomized (T) Dogs—The fatty acid content of the livers of dogs was measured at various intervals from 197 to 800 days after thyroidectomy (table 2). In 2 dogs that were sacrificed at 197 and 237 days, the fatty-acid content of their livers was not above normal. Five dogs were sacrificed between 365 and 387 days after excision of

this gland; in only one of them (T19) was the fatty-acid content in excess of 10 per cent. By the time 460 days had elapsed, however, the livers of 2 dogs contained about 20 per cent fatty acids. This concentration of fat was also found in the livers of 2 dogs that were sacrificed 800 days after thyroidectomy.

Hypophysectomized-Thyroidectomized (HT) Dogs—Fatty livers were found in 8 of the 9 dogs subjected to removal of both glands (table 3). In 3 of them the fatty-acid content of the liver was in excess of 30 per cent. In one (HT15) the fatty-acid content reached the astonishing value of 52.5 per cent.

TABLE 3. EFFECT OF HYPOPHYSECTOMY-THYROIDECTOMY ON THE LIVER LIPIDS OF THE DOG

Dog No.	Body weight				Period of observation		Liver	
	Initial	At hypophysectomy	At thyroidectomy	Final	After hypophysectomy	After thyroidectomy	Weight	Total fatty acids
	kg.	kg.	kg.	days	days	days	gms.	per cent
HT3	8.5	9.1	10.9	10.7	45	70	450	13.7
HT6	9.5	10.2	11.2	15.0	46	126	390	3.9
HT21	7.9	7.8	7.8	14.5	45	217	398	14.3
HT18	10.2	10.5	12.1	17.4	46	250	405	32.5
HT15	6.5	6.8	9.2	9.0	47	310	375	52.5
HT22	8.2	8.4	8.8	19.7	44	311	412	11.2
HT11	6.3	6.6	6.8	11.0	45	374	285	12.5
HT10	8.6	9.7	11.3	20.5	49	398	1455	30.5
HT8	8.0	8.8	10.3	16.5	47	419	989	16.2

The histological appearance of these livers has been described elsewhere (Chaikoff *et al.*, 1943). In addition to fattiness, an increased fibrous-tissue proliferation or frank cirrhosis was observed in many of the HT dogs.

DISCUSSION

In view of the well-known role of dietary factors in the prevention of fatty livers, it should be emphasized here that the thyroidectomized and thyroidectomized-hypophysectomized dogs were fed a diet high in protein. Each dog ingested daily approximately 300 gm. of lean meat. The development of fatty livers in these dogs. therefore, cannot be ascribed to their failure to ingest a diet adequate in lipotropic factors.

A marked gain in weight was observed in most of the HT dogs in which fatty livers were found. That this gain in weight, however, is not the cause of the fatty livers is borne out by the failure of the hypophysectomized dogs to develop fatty livers, though they too showed pronounced weight-gains during the period of observation. This view is reinforced by the finding (to be reported in the next paper) that choline prevented the accumulation of excessive amounts

of fat in the livers of the doubly operated dogs but did not prevent their gain in weight.

In view of the report of Graef *et al.* (1944), who concluded that fatty livers in their hypophysectomized dogs resulted from coincidental hypothalamic injury, serial sections of the hypothalamic regions of all our H and HT dogs were prepared and carefully examined. No evidence of injury was found in a single dog.

For reasons presented earlier in connection with blood-lipid changes found in T and HT dogs (Entenman *et al.*, 1942b), it would appear that some metabolic defect imposed by thyroidectomy is the primary cause of the fatty infiltration observed in the HT dogs. The more pronounced degree of fatty infiltration observed in the HT than in the T dogs is ascribed to the fact that the HT dogs were more effectively thyroidectomized.

SUMMARY

Hypophysectomy does not influence the fat content of the liver of the dog. Observations were extended for as long as 32 months after excision of the pituitary gland.

Thyroidectomized dogs fed a high protein diet develop fatty livers. Twenty per cent fatty acids were found in the livers of 2 dogs examined 460 days after thyroidectomy and in 2 dogs examined 800 days after it.

Fatty livers appear earlier and are more severe in dogs subjected to both hypophysectomy and thyroidectomy than in those in which only the thyroid has been excised.

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CHOLINE PREVENTS FATTY CHANGE AND CIRRHOSIS IN THE LIVERS OF DOGS SUBJECTED TO HYPOPHYSECTOMY AND THYROIDECTOMY[1]

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HAVING shown that fatty and cirrhotic livers occur in hypophysectomized-thyroidectomized (HT) dogs (Entenman *et al.*, 1948), we next directed our attention to explaining the mechanism of development of these lesions. It has been repeatedly shown that fatty livers can be produced by the administration of diets low in protein and other lipotropic factors. But the fatty livers of the HT dogs could not have resulted from a deficient intake of lipotropic factors, for, as already pointed out in the preceding paper, these dogs were fed a high protein (lean meat) diet adequate in all respects. It is shown here, however, that the addition of *free choline* to the diet of these doubly operated dogs not only completely prevents the accumulation of fat in their livers but results, as well, in an almost complete suppression of the hepatic fibrosis. This new finding suggests that the removal of both glands has in some way interfered with the availability or utilization of the lipotropic factors contained in the diet or has increased the animal's requirements for these substances. This study thus brings to light a new aspect of the action of ingested lipotropic foodstuffs, namely their dependence upon endocrine factors.

EXPERIMENTAL

For the first 2–3 weeks after its arrival at the laboratory, each dog was fed twice daily 15 gm. of lean meat per kilo body weight, 15 gm. of sucrose, one gram of Cogwill's salt mixture (Cogwill, 1923), and 2 gm. of bone ash. Three cc. of Sardilene and 5 cc. of Galen B were added to each morning meal; the vitamin contents of these 2 constituents have been recorded elswehere (preceding paper).

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Following a suitable control-period, the dogs were subjected to complete hypophysectomy (Dandy and Reichert, 1938). In the procedure used in this study no cautery was applied to the base of the brain. After a lapse of 34–60 days the dogs were thyroidectomized. One or two of the parathyroid glands were preserved with their blood supply intact. All other parathyroid tissue found was transplanted into neighboring muscle.

Following removal of both glands, the dogs were fed the diet described above *plus* one gram of choline chloride with each meal. Thus, throughout the entire period of observation after removal of both glands, each dog ingested, in addition to the regular diet, 2 gm. of choline chloride daily. Food not voluntarily eaten was force-fed.

To counteract the possible development of a parathyroid deficiency, each

TABLE 1. EFFECT OF CHOLINE ON LIVER FATTY ACIDS OF HYPOPHYSECTOMIZED-THYROIDECTOMIZED DOGS

Dog No.	Body weight				Period of observation		Liver	
	Initial	At hypophysectomy	At thyroidectomy	Final	After hypophysectomy	After thyroidectomy*	Weight	Total fatty acids
	kg.	kg.	kg.	kg.	days	days	gms.	per cent
HT28	5.0	5.2	5.6	9.0	45	782	488	2.5
HT29	9.4	9.6	9.3	14.2	34	782	502	4.3
HT30	6.2	6.2	6.9	11.1	34	782	320	2.4
HT31	6.0	6.3	5.6	8.1	60	720	210	2.2
HT33	6.0	6.2	6.3	10.8	60	720	245	2.9

* After thyroidectomy each dog received 2 grams of choline chloride daily.

dog was also fed during the first 2 weeks after thyroidectomy 5 gm. of calcium lactate and 100 cc. of whole milk.

At the end of the periods of observation (table 1) liver and blood were removed and their lipid contents determined by methods previously described (Chaikoff and Kaplan, 1934). Free cholesterol was determined in an acetone solution from which phospholipids had been precipitated. Tissues were also taken for histological examination. The completeness of hypophysectomy was established in all dogs at necropsy by serial sections of the base of the brain.

RESULTS

The Fat Content of the Liver—It was shown in the preceding paper that, by the time 799 days had elapsed after excision of the thyroid glands alone, dogs had developed fatty livers (Entenman *et al.*, 1948). Thus in 2 dogs that were sacrificed at this time-interval after thyroidectomy, 21.5 and 22.7 per cent fatty acids were found in mixed samples of their livers. The earliest interval after thyroidectomy when a fatty liver was found was 460 days. Fatty livers appear much earlier in the dogs subjected to hypophysectomy as well as to thyroidectomy. Thus, by the time 217 days had elapsed after excision of the second gland (thyroid) their livers contained more than 10 per cent fatty acids. From 30 to 50 per cent fatty acids were found in the livers

of 3 of the doubly operated dogs that were sacrificed from 250 to 398 days after thyroidectomy.

In the present study, the doubly operated (HT) dogs were sacrificed at 2 intervals, i.e., 720 and 780 days after the second gland (thyroid) had been excised (table 1). Throughout this entire period each dog ingested daily 2 gm. of choline chloride in addition to the regular lean-meat diet. The highest value for the fatty-acid content of the livers of 5 dogs so treated was 4.3 per cent.

In view of the fatty livers observed in thyroidectomized and in hypophysectomized-thyroidectomized dogs not treated with choline, it must be concluded that the ingestion of *free* choline prevents the

TABLE 2. PLASMA LIPIDS OF HYPOPHYSECTOMIZED-THYROIDECTOMIZED DOGS FED CHOLINE

Dog No.	After thyroidectomy	Total cholesterol	Phospholipid	Total fatty acids	Total lipid
	days	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.
HT28	782	181	825	2310	2491
HT29	782	192	1160	4540	4732
HT30	782	188	480	870	1058
HT31	720	438	575	835	1273
HT33	720	398	467	852	1319

accumulation of fat in the livers of the doubly operated dogs (table 1).

Blood Lipids—The plasma lipids of 5 HT dogs, which for 720 to 780 days after excision of the second gland received choline daily, are recorded in Table 2. The values for total lipids were well above 1000 mg. per cent. The concentrations of all constituents, namely total cholesterol, phospholipids, and total fatty-acids, were elevated. Thus the ingested choline, while it prevented completely the accumulation of extra fat in the liver, failed to prevent the rise in plasma lipids which we have previously recorded in HT dogs (Entenman *et al.*, 1942).

Body Weights—By the time they were sacrificed, all 5 of the choline-treated HT dogs showed considerable increases in their weights. This increase in weight was not unexpected, for, as already pointed out, the preoperative caloric intake was imposed upon dogs in which the metabolic rate had been reduced by excision of both glands (Entenman *et al.*, 1942). The prevention of fatty livers in the doubly operated dogs by the administered choline justifies the conclusion that weight gain *per se* was not responsible for the development of fatty livers in the HT dogs.

PATHOLOGICAL FINDINGS

In order to recognize the effects of choline upon the development of cirrhosis in the hypophysectomized-thyroidectomized dogs, it is necessary to compare the livers of 2 groups of dogs: those that ingested the 2 gm. of choline chloride daily for over 700 days and those that

received no choline. The livers of the latter will be described in detail elsewhere; hence the findings are merely summarized here for convenience of comparison.

a. *Hypophysectomized-Thyroidectomized Dogs Not Fed Choline*—The livers of 4 of the 9 doubly operated dogs (see preceding paper) were severely cirrhotic (fig. 4), while in the livers of 3 others fibrosis of varying extent and severity was present (fig. 2).

TABLE 3. PATHOLOGY OF CHOLINE-TREATED HYPOPHYSECTOMIZED-THYROIDECTOMIZED DOGS

Dog	Fat	"Plant-like" cells and hyalinization*	Hepatic fibrosis	Cirrhosis
HT28	0	4+†	2+‡	0
HT29	0	4+	0	0
HT30	0	2+	0	0
HT31	0	2+	0	0
HT33	0	2+	0	0

* See text for description and see figure 1.
† 2+ = About ¼ of lobules involved.
4+ = About ¾ of lobules involved.
‡ 2+ = Fine cellular connective tissue in about ¼ of the lobules.

The most common fibrotic changes (previously described as precirrhosis) consisted of a thickening of the reticulum around the large fat cells near the central or sublobular branches of the hepatic vein (fig. 2). This reticular thickening was associated with an infiltration of round cells, polymorphs, and fibroblasts. In the later stages these precirrhotic changes extended farther into the lobule and along the course of the large radicles of the hepatic vein, and subsequently the fibrous bands around the hepatic radicles of neighboring lobules joined one another. This fibrosis, commencing around the radicles of the hepatic vein, when more advanced, results in distortion of the lobular architecture and incorporation of the portal tracts in the fibrous tissue (fig. 4). Ultimately, scar tissue obliterates the area originally enclosed by the joining of the fibrous tissue which commenced around the central veins of adjacent lobules.

In addition to this fibrosis initiated around the smaller divisions of the hepatic veins, another form of fibrosis commencing around the small portal tracts was detected in two livers. In the livers of two dogs

EXPLANTION OF PLATE I

FIG. 1, Dog HT30, fed choline. Note the very sharp cell walls and the granularity of the liver cell protoplasm ("plant-like" cells). The eosinophilic hyalinization of the liver cells associated with nuclear hyperchromasia is well shown. Round cell foci such as depicted here (top left corner) are common. Hematoxylin and Eosin ×225.

FIG. 2. Dog HT22, not fed choline. The pericentral fatty change is clearly shown together with the edema. cell infiltration, and early fibrosis (precirrhosis) around the central or sublobular vein. The small portal tract, below and to the right of the above mentioned lesion, is normal. Note also the early extension of the fibrosis along the fatty zones. Hematoxylin and Eosin ×70.

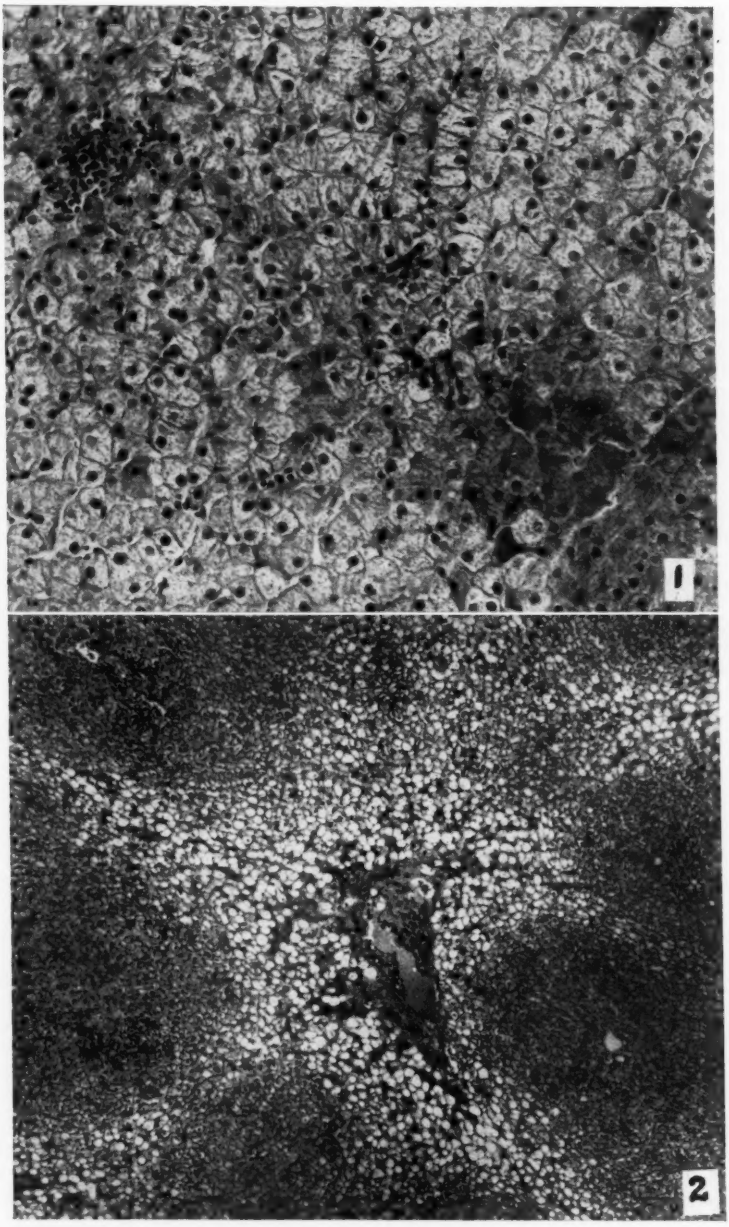

PLATE I

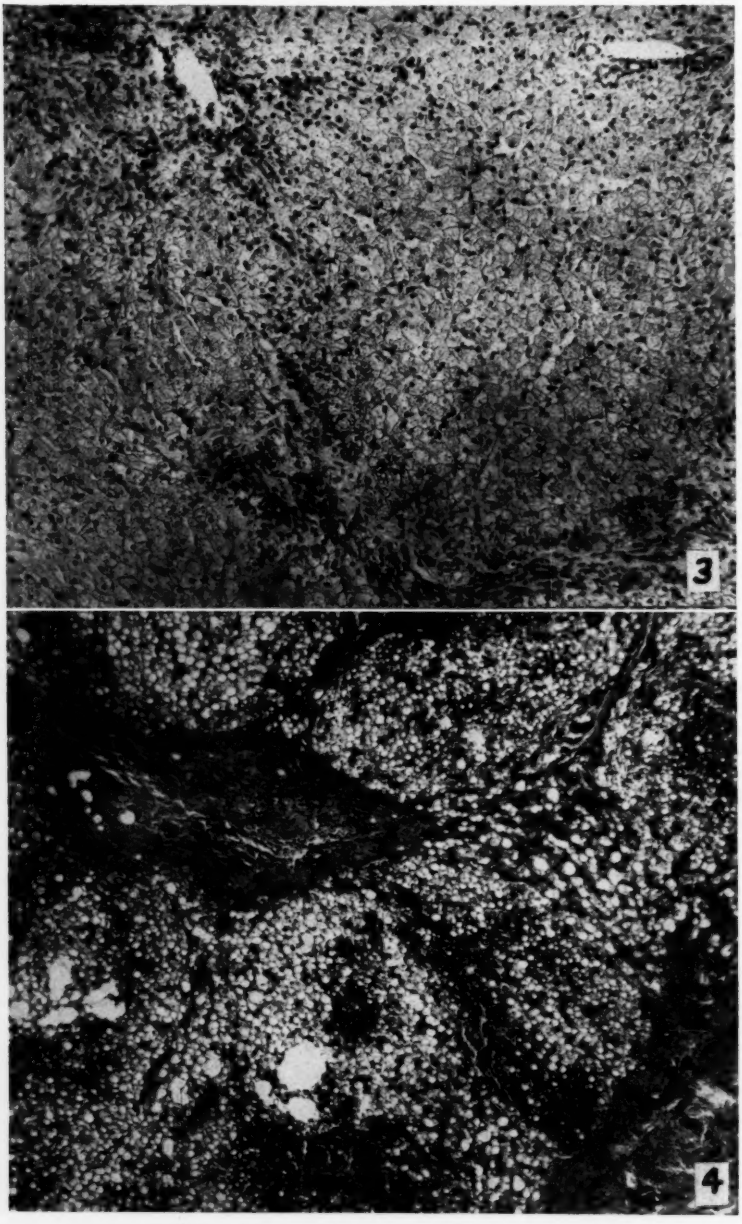

PLATE II

both periportal and perihepatic vein fibrosis were apparently progressing simultaneously.

b. *Choline-Treated Hypophysectomized-Thyroidectomized Dogs*—In only one of the 5 dogs treated with choline after ablation of both pituitary and thyroid glands, was any significant change observed in the liver. In this single animal changes regarded as indicative of early fibrosis were seen around many of the central veins, with complete absence of fat (fig. 3).

Neither fatty change nor evidence of fibrosis was detectable in the livers of any of the other dogs in this series. Apart from two peculiar changes in the cytoplasm of the liver cells and an unusual prominence of their cell walls, these livers could easily be taken for normal.

The two cytoplasmic changes in the liver cells to which we wish to draw attention are, firstly, a peculiar clumping of the chondriosomes which lie in a nonstaining cell sap and, secondly, an intense eosinophilia with hyalinization of scattered patches of the liver cells (fig. 1). In the former reaction the cell membranes of the affected liver cells are unusually thick and prominent ("plant-like" cells). These cannot be regarded as features of normal liver cells, but the significance of these changes is at present obscure.

However, despite these changes in the liver cells, it is clear from a comparison of figs. 1 and 3 with figs. 2 and 4 that the administration of choline has had a most remarkable effect in preventing the gross fatty changes so common in the untreated dogs deprived of both thyroid and pituitary glands. Moreover, the choline also diminished the incidence of cirrhosis and, in addition, in the one dog with precirrhosis, its ingestion limited the extent and rate of progress of fibrosis.

DISCUSSION

A clue to the mechanism by which fatty livers develop in hypophysectomized-thyroidectomized dogs is provided by the observation that supplementation of their diets with 2 gm. daily of *free* choline chloride prevented this change. Each dog ingested daily from 200–300 gm. of lean meat; the lipotrope content of this amount of meat is more than enough to prevent fatty livers in normal dogs. The fact, therefore, that choline prevented fatty change in the liver means either 1) that the release of bound methionine and bound choline from dietary

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EXPLANATION OF PLATE II

FIG. 3. Dog HT28, fed choline. Note the cell infiltration and early fibrosis around both the small portal tract above and the centrolobular area below and to the right. This is the most severe degree of fibrosis encountered in the choline-treated dogs and is regarded as a very mild lesion. Hematoxylin and Eosin×110.

FIG. 4, Dog HT22, not fed choline. Note the severe fatty change, the obliteration of two lobules by fibrous tissue, and the early intralobular fibrosis between these two scars and towards the lower left. This severity of cirrhosis was encountered in several of the HT dogs. Comparison of this figure with fig. 2 above (both sections from different lobes of the same liver) reveals the extent of variation in reactivity which may occur in different lobes of a single liver. Hematoxylin and Eosin ×60.

constituents in the intestinal tract of the doubly operated dog is interfered with, or 2) the absorption of these substances is defective, or 3) that an interference in the utilization of lipotropic factors has occurred. As already shown, fatty livers do not develop in hypophysectomized dogs fed the stock diet but do occur after thyroidectomy, with or without hypophysectomy. This indicates that the effectiveness of the lipotropic constituents of a normal diet is, in part, dependent on the action of the thyroid hormone.

The action of choline observed here in preventing fatty infiltration and cirrhosis in the livers of dogs subjected to hypophysectomy and thyroidectomy should not be confused with a similar action of choline in rats fed a low-protein, low-choline diet (Blumberg and McCollum, 1941; Lillie *et al.*, 1942; Engel, 1943; György, 1944). In the latter, the preventive action of choline upon both the fatty liver and cirrhosis was to be expected, since the rats were fed a diet deficient in lipotropic factors. This was not so for the choline effects reported here; choline completely prevented fatty livers and suppressed fibrosis in hypophysectomized-thyroidectomized dogs which throughout the experimental periods were fed a diet adequate in lipotropic factors as well as in other respects.

SUMMARY

The development of fatty livers previously reported in hypophysectomized-thyroidectomized dogs fed a high-protein diet can be prevented by the daily administration of 2 gm. of *free* choline chloride. Choline failed, however, to prevent the rise of the blood lipids in these dogs.

Choline also significantly suppressed or prevented the onset and development of hepatic fibrosis in these doubly operated dogs.

These results indicate that in the dog deprived of thyroid and pituitary glands there develops either 1) an interference in the availability or utilization of the lipotropic factors present in a meat-sucrose diet supplemented with vitamins and minerals or 2) an increased need for these substances.

The evidence obtained here indicates that the effectiveness of the lipotropic constituents of a normal diet is, in part, dependent on the action of the thyroid hormone.

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EFFECT OF THE DIETARY PROTEIN CONCENTRATION UPON THE SECRETION OF ADRENOCORTICOTROPHIN

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MONTREAL

IT HAS been previously reported (Dontigny, Hay, Prado and Selye, 1947) that animals chronically treated with large doses of crude anterior pituitary preparations become hypertensive if they are simultaneously fed a high (30%) protein diet. At that time attention was called to the fact that the hypertension was accompanied by a marked adrenal enlargement, while rats treated with the same pituitary preparation but receiving a low (15%) protein diet maintained a normal blood pressure and showed but slight adrenal stimulation. Subsequently, Prado, Dontigny and Selye (1947) showed that the concentration of protein in the diet does not affect the hypertension produced by desoxycorticosterone acetate overdosage. In order to give a plausible explanation to these observations, it was considered that the dietary protein could affect hormonal hypertension either by enhancing the response of the adrenal to adrenocorticotrophin or by increasing corticotrophin secretion by the anterior pituitary.

In order to gain further information concerning this point, we have studied the effect of the dietary protein concentration upon the responsiveness to stress of the hypophyseo-adrenal system. Two types of experiments have been performed: I, those based on the effect of injected corticotrophin upon the adrenal ascorbic acid; II, those concerning the compensatory adrenal hypertrophy (due to endogenous corticotrophin formation), as influenced by dietary protein.

I. ADRENAL ASCORBIC ACID

Methods and experiments. The suprarenal response to exogenous corticotrophin was estimated, using as a criterion, the decrease of the adrenal ascorbic acid concentration as described by Sayers, Sayers, Liang and Long (1945) and by Sayers and Sayers (1946).

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This sensitive test has also been shown to be very useful in the study of endogenous corticotrophin activity of normal animals (Sayers and Sayers, 1947). In the present work, it has been used for both these purposes.

The adrenal ascorbic acid was determined by Carruthers' (1942) method. We slightly simplified it by estimating the ascorbic acid from a standard curve prepared from known amounts of vitamin C, instead of deriving it from the colorimetric readings before and after reduction of the standard 2, 6-dichlorophenolindophenol. The reliability of the method did not seem to be, in any way, impaired by this modification.

Four groups of male, adult, albino rats were fed the experimental diets (Table 1) during a period of three weeks. Groups. 1 and 3 received the 15%-

TABLE 1. COMPOSITION OF TWO BASIC SYNTHETIC DIETS
(parts per cent)

Diets		I	II
	Casein	15	30
	Corn Starch	78	63
	Fat	1	1
	Cod liver oil	1	1
	Bulk	1	1
	Mineral mixture	4	4
Supplements:	Thiamine chloride	0.8 mg./100 gm. diet	
	Riboflavin	0.8 mg./100 gm. diet	
	Pyridoxine	0.8 mg./100 gm. diet	
	Ca pantothenate	4.0 mg./100 gm. diet	
	Nicotinic acid	1.0 mg./100 gm. diet	
	Choline chloride	100.0 mg./100 gm. diet	
	α-Tocopherol acetate	10.0 mg./rat/weekly	

casein-diet (Diet I), while groups 2 and 4 were fed a 30%-casein-ration (Diet II). At the end of the preparatory feeding period and after fasting the animals for 24 hours, groups 1 and 2 were exposed to a temperature of 4 ± 1°C. for one hour and the remaining two groups were kept at room temperature as controls. All animals were then killed with chloroform, and the ascorbic acid concentration of the adrenal glands was determined.

The same experiment was repeated subjecting the animals to a greater degree of stress (0 ± 1°C.).

Another experiment was then performed in order to ascertain the effect of the dietary protein concentration on the adrenal response to a given amount of exogenous corticotrophin. Two groups of adult, male, albino rats (70 to 85 gm.) were respectively fed diets I and II during three weeks at the end of which time they were hypophysectomized. Twenty four hours later, the right adrenal was removed under nembutal anesthesia, and immediately after, 10γ of corticotrophin was intravenously injected. One hour later the contralateral gland was also removed and the ascorbic acid concentration of each gland was separately determined. Care was taken to prepare the extract of each gland as it was extirpated. The cattle pituitary corticotrophin used was prepared by Fishman's (1947) method.

Results. Perusal of Table 2 reveals that exposure to cold caused a greater reduction of the adrenal ascorbic-acid concentration in ani-

mals receiving the high-protein-diet, than in rats fed the low-protein-diet. If the decrease in adrenal ascorbic acid is expressed as the percentage of the control value, the difference between each exposed group and its respective control is of the same order of magnitude in both experiments, 1 and 2, in spite of the admittedly, unexplained, low control values observed in the first experiment. These results indicate that the hypophyseo-adrenal system of animals receiving a

TABLE 2. INFLUENCE OF DIET UPON THE RESPONSE OF THE HYPOPHYSEO-ADRENAL SYSTEM TO COLD

Group	Treatment	No. of rats	Diet	Adrenal ascorbic acid mg./100 gm. fresh tissue			
				Average ± S.E.	Decrease from control level		Decrease in percentage
1	Cold (exposed to 4 ± 1° C. for 1 hr. before killing)	5	I	247 ± 5.2	32 ± 13.3		11.5
		4	II	212 ± 15.0	73 ± 15.6		25.6
3	Unexposed controls	5	I	279 ± 12.2			
4		3	II	285 ± 29.2			
1	Cold (exposed to 0 ± 1° C. for 1 hr. before killing)	7	I	326 ± 5.5	48 ± 17.0		12.8
2		6	II	286 ± 8.7	148 ± 25.2		34.1
3	Unexposed controls	5	I	374 ± 16.0			
4		6	II	434 ± 23.8			
				Before corticotrophin injection (Left adrenal)	After corticotrophin injection (Right adrenal)	Decrease after injection	
1	Hypophysectomized; 10γ corticotrophin (after unilateral adrenalectomy and 1 hr. before killing)	6	I	450 ± 10.2	325 ± 18.8	135 ± 16.2	30
		11	II	416 ± 18.8	320 ± 16.4	96 ± 15.4	23

S.E. = Standard Error.

high-protein-diet gives a greater response to stress than that of animals fed a low protein diet.

These first two experiments, however, did not establish which gland of the hypophyseo-adrenal system was rendered more responsive to stress by the diet. It was in order to study the effect of the diet on the corticotrophin responsiveness of the adrenal itself that we injected the same amount of corticotrophin to hypophysectomized rats receiving one or the other of the two experimental diets. The results of experiment 3 (Table 2) show the decreases in adrenal ascorbic acid induced by the injection of 10γ of corticotrophin into two groups of animals pretreated with diets I and II. As the decreases observed were not significantly different ($p = 0.1$) it was concluded that the dietary protein concentration did not modify the adrenal response to exogenous corticotrophin.

II. COMPENSATORY HYPERTROPHY OF THE ADRENAL

Methods and experiments. The above results indicated that corticotrophin secretion is affected by the dietary protein concentration even in acute experiments, but we felt that a clearer effect might be observed in a long-term experiment. Considering that in the ascorbic acid test the actual period of

pituitary stimulation is limited to one hour, we thought that a study of the degree of adrenal compensatory hypertrophy, as affected by the dietary protein concentration, might furnish additional evidence of differences in pituitary activity.

In the first experiment, forty male albino rats (80–100 gm.) were divided into four groups: the first two groups received diet I (15% casein), and the last two, diet II (30% casein). After one week, the left adrenal was removed in all animals of groups 2 and 4, while groups 1 and 3 were kept as controls. Five animals of each group were killed on the fifth day after adrenalectomy

TABLE 3. EFFECT OF THE DIETARY PROTEIN CONCENTRATION ON THE DEGREE OF ADRENAL COMPENSATORY HYPERTROPHY

(Average and Standard Errors)

Diets	Operation	No. rats	Autopsy days after adr-ect.	Final body weight gm.	Right adrenal weight mg.	Adrenal weight mg./100 gm. of body weight
I	Control	4	5 days	139 ±3.8	10.0 ±0.81	7.2
	Adr-ect.*	5		128 ±4.9	10.2 ±0.39	7.9
II	Control	4		145 ±5.8	10.9 ±0.76	7.5
	Adr-ect.	4		130 ±6.6	11.6 ±0.91	9.0
I	Control	11	10 days	155 ±3.9	9.9 ±0.36	6.4
	Adr-ect.	11		154 ±5.6	11.7 ±0.63	7.2
II	Control	10		178 ±4.1	9.8 ±0.27	5.6
	Adr-ect.	13		173 ±2.3	13.7 ±0.50	8.1
I	Control	10	15 days	179 ±3.9	9.7 ±0.23	5.4
	Adr-ect.	9		168 ±4.4	10.6 ±0.46	6.4
II	Control	8		187 ±3.9	9.2 ±0.32	4.9
	Adr-ect.	9		170 ±5.2**	13.3 ±0.45	7.8

* Adr-ect. = adrenalectomized.
** = Initial body weight proportionally smaller.

Significance of differences in average adrenal weight

5 days	10.2 ±0.39 11.6 ±0.99	p = 0.2
10 days	11.7 ±0.63 13.7 ±0.50	p = 0.02
15 days	10.6 ±0.46 13.3 ±0.45	p < 0.01

and the other animals on the tenth day. The remaining adrenals in the operated groups and the right adrenals of the control groups were fixed in "Suza" for 24 hours, dissected and weighed on an analytical balance. This experiment was repeated twice: the second time all the animals were sacrificed 10 days after the unilateral adrenalectomy and in the third experiment, 15 days after the operation.

Results. As may be seen from Table 3, the degree of adrenal compensatory hypertrophy was markedly affected by the composition of the diet. The proliferation of the remaining adrenal gland was more rapid in the animals receiving diet II (30% casein) than in those fed

diet I (15% casein). This difference is significant ten or fifteen days following the operation whether calculated from the absolute or from the relative weights of the glands (mg. adrenal tissue/100 gm. body weight). The regeneration curves shown in Fig. 1 are obtained by plotting the percentage increases in relative adrenal weights against the experimental periods following unilateral adrenalectomy. It was not established whether the regeneration curve of the adrenal tissue of the animals receiving the 15%-casein-diet would eventually reach the curve of the 30%-protein-group or whether it would permanently remain at a lower level.

INFLUENCE OF THE DIETARY PROTEIN CONCENTRATION UPON THE REGENERATION-RATE OF ADRENAL TISSUE.

(Increases are expressed as % of the initial values in mg. adrenal tissue/100 gm. body weight. Time in days after unilateral adrenalectomy.)

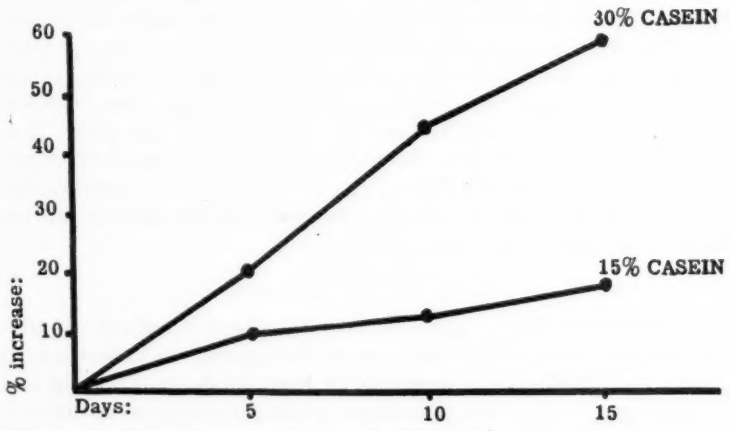

FIG. 1.

DISCUSSION

The evidence presented in this paper, derived both from Sayers' ascorbic acid test and from the regeneration of adreno-cortical tissue, seems to indicate that, under our experimental conditions, the dietary protein concentration affects the rate of corticotrophin secretion by the anterior pituitary. It is not possible to conclude with certainty from the available data whether the formation or merely the discharge of preformed hormone is altered by the diet. It would be difficult, however, to explain the more rapid regeneration of adrenal tissue induced by a higher protein diet in terms of increased discharge only, hence we are inclined to believe that the rate of secretion is increased. A third possibility, though quite hypothetical, is more difficult to eliminate, namely that the high-protein diet stimulates the elaboration by the hypophysis of corticotrophin-synergizing material.

No difference was found between the adrenals in the control groups receiving diet I (15% protein) and diet II (30% protein) respectively either in the ascorbic acid tests or in the compensatory hypertrophy experiments. This indicates that the diets used, do not—in themselves—induce a greater corticotrophin secretion. Only if the anterior pituitary is stimulated to increased corticotrophin production (by cold or by a partial corticoid insufficiency), does a difference appear between the animals receiving the high and the low-protein-rations.

The results obtained with the ascorbic acid test in hypophysectomized rats show that the adrenal cortex response to a known amount of corticotrophin is not modified by the diet. In this respect our observations confirm those of Ingle, Prestrud, Li and Evans (1947), regarding the effect of diet upon corticotrophin activity in intact rats. It is difficult to reconcile our results, however, with those of Benua and Howard (1945), who were unable to detect any effect of the dietary protein concentration upon the degree of adrenal compensatory hypertrophy. In the diets used by those authors, 66.8 to 89.3% of the total caloric value was derived from proteins; perhaps these concentrations were excessive and the diet as a whole too abnormal. It is noteworthy that the body growth of the unilaterally-adrenalectomized animals used in Benua and Howard's experiments, was significantly retarded; an effect which we did not observe with the dietary protein concentrations used in the course of this work.

SUMMARY AND CONCLUSIONS

Experiments on the rat indicate that the adrenal-cortical stimulation elicited by stress (e.g., cold) or by unilateral adrenalectomy is greater in animals kept on high (30%) than in those receiving low (15%) protein diets. Such a difference in the dietary protein concentration does not exert any significant effect upon the adrenal under basic conditions in normal animals, nor does it demonstrably affect the adrenal response of hypophysectomized rats to exogenous corticotrophin.

It is concluded that diets rich in protein do not sensitize the adrenal to corticotrophin, nor do they normally increase corticotrophin secretion by the pituitary. Under certain conditions, however, when adrenal growth is stimulated beyond normal, high-protein diets further augment corticotrophic response, either by increasing corticotrophin production or by eliciting the elaboration of some corticotrophin-synergizing hypophyseal principle.

ACKNOWLEDGMENTS

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NOTES AND COMMENTS

URINARY STEROID EXCRETION PATTERNS OF TWO PSEUDOHERMAPHRODITES

In the examination of urine from two pseudohermaphrodites, we have observed by simple means consistent differences in the steroid excretion products which seem worth noting. By the Venning (1937) method, glucuronide containing a large amount of ketosteroid (Strickler *et al.* 1943)[1] is obtained from the urine of case J. W.,[2] while the second case (B. A. R.)[3] if indeed she excretes this same material, must do so in much smaller proportion. This is shown by comparison of the hydrolysis products and by our use of the Tollens test. The two cases also differ in that free steroid may usually be obtained by alcohol-water precipitation from the acetone-soluble residue after removal of glucuronide from extracts of urine of case B. A. R., while none was found in eleven different collections of urine from case J. W. Some data on (17-)ketosteroid excretion (as estimated by the Zimmermann reaction) of case J. W. have already been published (Strickler *et al.* 1941). In general, the 24-hour excretion of (17-)ketosteroids (80 to 220 mg. as androsterone) by case B. A. R. is about twice as high as that of case J. W. by our method. Case J. W. has been receiving endocrine therapy, mainly diethylstilbestrol, at various times, but examination of the accumulated data on excretion of glucuronide and ketosteroids has revealed no correlation with medication. (However, the amount of diethylstilbestrol given was aways considerably less than the quantities given by Talbot *et al.* (1942) to those cases in which they obtained lowering of ketosteroid excretion.) The second case never had received any endocrine medication. No tumor could be found in either case on laparotomy.

Further details of the differentiation by simple chemical means are given below.

Experimental

The crude glucuronide obtained from the urine of case J. W. ranged from 40 to 100 mg. per 24 hours; that from case B. A. R. was usually more resinous, and varied widely in amount up to 63 mg. per 24 hours. In one series of collections of urine from the latter case, 110 mg. very crude glucuronide and 270 mg. "precipitable free steroid" were obtained from 13½ liters (eleven 24-hour specimens). In practically all of the eleven specimens, if the glucuronide excretion was relatively high, the "precipitable free steroid" was low and vice-versa. To see whether the low glucuronide yields could be increased by the use of lower temperatures in the isolation procedure, urine was in one instance worked up both by the method of Talbot and Eitingon

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[1] Identified by Dr. H. L. Mason (Mayo Clinic) as pregnane-3 (α), 17-diol-20-one. To be published.

[2] Case of Dr. John W. Shirer.

[3] Case of Dr. Joseph Hepp.

(1944) at or below room temperature and the regular Venning method. Little conjugate was obtained, and a good yield of "precipitable free steroid," indicating splitting before extraction. This occurred despite use of tricresol plus butyl alchohol or the latter alone for preservation of the urine.

The glucuronide of case B. A. R. gives the abnormal behavior in the Tollens test shown by the glucuronide of case J. W. (Strickler *et al.* 1943), insofar as the aqueous phase is concerned, but it differs in that there is considerable color in the ether phase, comparable to that obtained with the NaPG run simultaneously. A recrystallized sample of the glucuronide (9.1 mg. out of 22.7 mg.) melted at 261–263° with decomposition. The rest of this plus the mother liquors yielded on acid hydrolysis a material which melted after recrystallizing from acetone at 236–237°[4] with preliminary sintering. (The highest melting point found for the easily obtained ketosteroid of case J. W. was 213°.) One lot of the "precipitable free steroid" after recrystallization from acetone melted at 241–245°. Yields on recrystallization of this material from acetone were low. Mixed melting point determinations of this "free steroid" sample gave respectively 241–243° and 238.5–241° with pregnanediol of pregnancy urine (m. pt. 237–238°) and the steroid from the glucuronide. This material did not give any color in the Tollens test (aqueous phase) as did the ketosteroid of case J. W. Androsterone[5] did not give the test either.

It should be noted that precipitation of the "precipitable free steroid" is incomplete from 1:4 alcohol-water, unlike pregnanediol of pregnancy, so that the ratio had to be increased to 1:5 or 1:5.5.

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[4] Fisher-Johns melting point apparatus.

[5] Furnished by Dr. C. Scholz of the Ciba Pharmaceutical Co.

[6] Present address Westinghouse Research Laboratories, East Pittsburgh, Pennsylvania.

INCREASED GLYCONEOGENETIC FACTOR PRODUCTION AFTER ADRENAL ENUCLEATION.[1]

IT HAS recently been shown (Hartman, Brownell and Knouff, 1947) that, following enucleation of both adrenals in the mouse, there is a marked increase in the output of fat factor from the adrenal. In the present study we have determined the production of the glyconeogenetic factor following enucleation and find that it also is increased considerably. The maximum production is reached at a much later time than that for the fat factor.

METHODS

Male albino mice of the Harlan strain, 10 to 14 weeks of age, were employed. Before operating, these had been kept in the laboratory for one week or longer on a Purina chow diet. Enucleation was performed as described in the previous communication.

To determine the amount of glyconeogenetic factor being produced, the animals were starved for 24 hours. After anesthetization with nembutal, the liver was quickly removed and its glycogen content determined by the method of Good, Somogyi and Kramer (1933).

RESULTS

The results are shown in the accompanying figure and table (Fig. 1; Table 1). The number of animals used on each point is indicated in the table.

TABLE 1. LIVER GLYCOGEN VALUES

Condition of animals	No. of animals	Mean per cent	"T" value
Normal	39	.1107 ± .07*	
Adrenalectomized	30	.0262 ± .0041	
2 days enucleated	15	.0466 ± .023	
9 days enucleated	33	.078 ± .072	
13 days enucleated	50	.162 ± .011	4.47
22 days enucleated	17	.187 ± .128	2.21
29 days enucleated	50	.403 ± .304	6.50
36 days enucleated	19	.158 ± .131	1.47
50 days enucleated	10	.171 ± .178	0.99

* Standard deviation.

The individual values for the adrenalectomized animals are not shown, since they were so grouped that space did not permit (see Fig. 1). There were 30 of these, the values of liver glycogen ranging from 0.024 to 0.053 per cent. The values after two days' enucleation were in the range of the adrenalectomized animals. However, at nine days, there was an increase over the adrenalectomized level, and by the thirteenth day the glycogen content was

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considerably above that for normal animals. The mean liver glycogen value for the fifty animals used was 0.162 per cent, which gives the highly significant t value of 4.47. At 22 days, the average liver glycogen as determined in 17 animals was even higher, 0.187 per cent. However, the small number of determinations and large standard deviation made $t=2.21$, which is of only probable significance. On the twenty-ninth day, the liver glycogen had reached the peak, which was three times that for normal animals, and showed

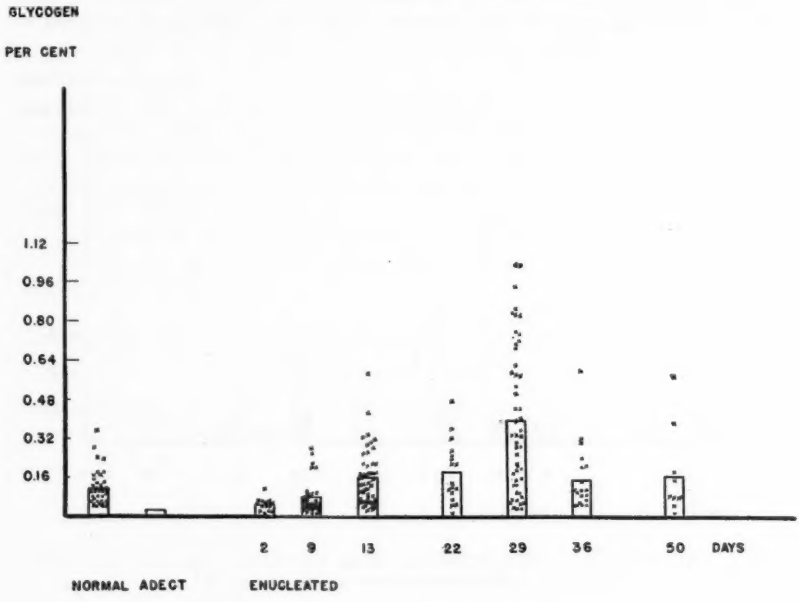

FIG. 1. Liver glycogen values after 24 hr. starvation.

the greatest spread of values. However, the mean of 0.403 per cent is highly significant, since $t=6.5$. It should also be noted that two-thirds of the animals possessed liver glycogens far above normal. By the thirty-sixth day there was a sharp decline to a mean value of 0.158 per cent. Although the average was well above normal, again the small number of animals and large standard deviation make the variation from the control level statistically insignificant, with a t value of 1.47. A higher glycogen level was maintained for at least 50 days. Ten determinations made at this point showed a mean value of 0.171 per cent, which is 65 per cent above the mean for normal controls. More animals would have to be run to determine the statistical significance.

The output of glyconeogenetic factor has been compared with that for the fat factor (1) in Figure 2. It will be noted that maxima for the two factors occur at widely different times, that for fat appearing at only 9 days after enucleation while that for carbohydrate does not appear until the twenty-ninth day.

DISCUSSION

The increase in output of the glyconeogenetic factor after enucleation resembles that of the fat factor (1) in two ways: first, the maximum is far above normal, three times in this instance (if the glycogen deposition is in proportion to the hormone output); and second, a higher than normal level is maintained for several weeks following the operation. However, the significant difference as already indicated is the occurrence of widely separated maxima. The high point for the fat factor occurred at nine days, while that for the glyconeogenetic factor occurred at 29 days. There also appeared to

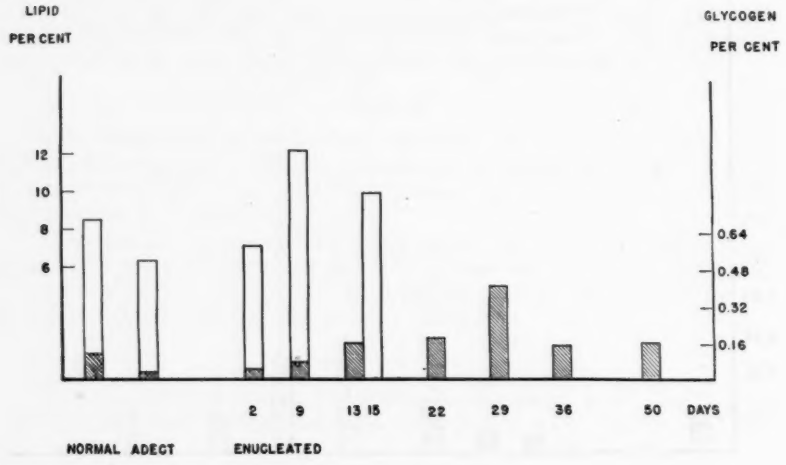

FIG. 2. Liver lipid and glycogen values after 24 hr. starvation, compared. Liver lipid values are taken from a previous publication (1).

be a more abrupt rise and fall to and from the maximum for the latter factor than for that for fat.

The obvious explanation of the increased hormone production after enucleation is that the sudden reduction of adrenal hormone output permits the pituitary to secrete more adrenocorticotrophic hormone which, in turn, stimulates the remaining cortical tissue to increased performance. As in the fat factor, it is difficult to understand why the increase in glyconeogenetic factor does not produce earlier inhibition of the pituitary than is apparent from the data.

The great range of values which occurs in all enucleated groups, and especially at the 29-day period, is noteworthy. We know from our own adrenal weight determinations after enucleation, in other studies not published, that the amount of regeneration varies greatly fron ome individual to another. Moreover, in some instances one adrenal may disappear entirely. Two explanations may be offered for the variation in glyconeogenetic response; either the pituitary fails to increase its adrenocorticotrophic hormone output as much in some animals as in others when adrenal tissue is reduced, or an unknown factor prevents the optimum regenerative response in some ani-

mals but not in others. Although a few cells can produce a very great increase in glyconeogenetic factor there is probably a limit to their output.

The occurrence of widely different maxima in point of time for the fat and carbohydrate factors indicates distinct hormones. Moreover it suggests two adrenocorticotrophic hormones.

Efforts are being made to separate these hormones from the respective glands.

SUMMARY

The amount of glycogen in starved mice was determined in normal and adrenalectomized animals and in animals whose adrenals had been previously enucleated.

Within two weeks after enucleation of both adrenals, the output of glyconeogenetic factor was much above normal. The maximum output, which was three times normal, was reached at about 29 days. Following this there was an abrupt fall to a level above normal which was maintained for at least three weeks.

It is pointed out that the maxima for the production of fat and glyconeogenetic factors occur at widely different times, which would indicate distinct hormones.

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